VSSA Summer Symposium Abstracts

Summer 2023

Thursday August 3
1-3 pm
Langford Lobby

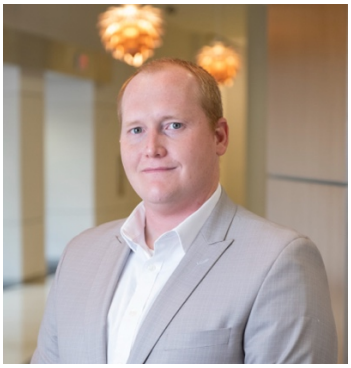
We want to thank everyone who participated in supporting the research summer experience for our interns: the principle investigators and mentors, lab personnel and staff, and program directors and our weekly speakers. It truly takes everyone to make the Vanderbilt Summer Science Academy happen.

Please join us in celebrating the accomplishments of the research interns, and visit the Poster Session to learn what they have done this summer.

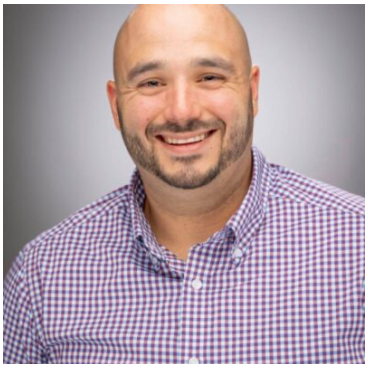
Sincerely,
The VSSA Team

Stephanie Richards, Ph.D.,
Director

Angel Gaither,
Program Manager



Aaron Howard,
Program Coordinator



RC Stabile, Ed.D.,
Associate Director of Well-being

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13	Jennifer Diaz Sales	Berea College	Aspirnaut
11	Bailey Groff	Elizabethtown College	Aspirnaut
44	Oscar Hanson	Berea College	Aspirnaut
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38	Kaziah Vaughn	University of Vermont	Aspirnaut
42	Shaelyn Walker	Duquesne University	Aspirnaut
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17	Jaela G. Melton	North Carolina Agricultural and Technical State University	MSTP Summer Research Program
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8	Jessica M. Bedenbaugh	Tennessee State University	PAECER
35	Selam Desta	Howard University	PAECER
53	Kate Ifeoma Ejimogu	University of Maryland Baltimore County	PAECER

Poster Number	Name	Home Institution	Program
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33	Channita Keuk	Sewanee: The University of the South	V-SURE
26	Sarah Livingston	Sewanee: The University of the South	V-SURE
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Participants and Programs, Session 1

1:00 – 2:00 pm

Poster #	Presenter	Program Affiliation
25	Nadine Abazie, Howard University	Aspirnaut
23	Adrian Aligwekwe, North Carolina State University	MSTP Summer Research Program
55	Hugo Arce-Santamaria, Berea College	Aspirnaut
41	Elijah Eshaun Burks, Tulane University	MSTP Summer Research Program
31	Sarah Cook, Yale University	UCRIP
21	JoAnna Dennis, Alabama Agricultural and Mechanical University	Aspirnaut
35	Selam Desta, Howard University	PAECER
13	Jennifer Diaz Sales, Berea College	Aspirnaut
53	Kate Ifeoma Ejimogu, University of Maryland Baltimore County	PAECER
1	Sierra Nichole Foster, Spelman College	PAECER
15	Maci Fulton, Florida Agricultural and Mechanical University	PAECER
49	Leila Ghaffari, University of Maryland, Baltimore County	MSTP Summer Research Program
11	Bailey Groff, Elizabethtown College	Aspirnaut
45	Kayla Hardrick, Miles College	Aspirnaut
9	Briana Harness, Western Kentucky University	MSTP Summer Research Program
7	Joseph Girgis Helmy, Lipscomb University	UCRIP
43	Gordina Princess Hodibert, University of Virginia	PAECER
29	Ian Johnson, Colorado College	UCRIP
51	Madeline Jones, Lawrence University	UCRIP
47	Hira Karim, Berea College	Aspirnaut
33	Channita Keuk, Sewanee: The University of the South	V-SURE
5	Ellie Kowitz, Lipscomb University	UCRIP
61	Andrea Mancia, Emory University	MSTP Summer Research Program
17	Jaela G. Melton, North Carolina Agricultural and Technical State University	MSTP Summer Research Program
27	Hailey Mackenzie Mullins, Spelman College	PAECER
3	Suzanne Alicia Navarro, Vanderbilt University	PAECER
37	LilyJasmine Notice, Oakwood University	Aspirnaut
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19	Stetler Tanner, Brigham Young University	Independent Research Intern
59	Logan Tsukiyama, Case Western Reserve University	VUSE
57	Kaiwen Zheng, Sewanee: The University of the South	V-SURE

Participants and Programs, Session 2

2:00 – 3:00 pm

Poster #	Presenter	Program Affiliation
12	Matthew Ahlers, Duke University	UCRIP
58	Christopher Thomas Altamura, Stony Brook University	PAECER
56	Chrystal Omonzele Aluya, Harvard University	UCRIP
8	Jessica M. Bedenbaugh, Tennessee State University	PAECER
30	Alexandria Bustabad, University of South Florida	Vanderbilt Diabetes Summer Research Program
40	Miles Carter, New York University	BP-ENDURE
52	Yuna Chung, University of Richmond	UCRIP
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22	Isabelle Cruz Hill, Howard University	PAECER
48	Renaya Imani Kelly, North Carolina Agricultural and Technical State University	PAECER
34	Marina Saber Khalil, Middle Tennessee State University	UCRIP
36	TyJanae Livers, Miles College	PAECER
26	Sarah Livingston, Sewanee: The University of the South	V-SURE
54	Harrison C. Lucas, Brandeis University	UCRIP
18	Nawshin Maleeha, Macaulay Honors College at CUNY Hunter College	BP-ENDURE
20	Maylyn Mei, Hunter College	BP-ENDURE
12	Sherwin Samuel Newton, Baylor University	UCRIP
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6	Qiana Jianni Tanael Williams, Miles College	PAECER
14	Ryan Xavier, The University of the South	V-SURE
32	Madison Yarbrough, University of Central Arkansas	Aspirnaut

ABSTRACTS

1. Regeneration of Renal Papilla after Reversal of Unilateral Ureteral Obstruction

Presenter: Sierra Nichole Foster, Spelman College

Program: PAECER

Principle Investigator: Mark de Caestecker, MBBS, Ph.D., Nephrology Department

Additional Project Authors: William Snyder, Kelly Clouthier, Maya Brewer, Rachel Delgado, Charlene Finney

Abstract:

Introduction: Obstructive uropathy is caused by blockage of urine flow in the bladder or ureters. Even after the reversal of blockage, this can lead to long-term damage in the renal papilla, which regulates urine concentration. This is mimicked in mouse models of reversible unilateral ureteral obstruction (RUUO). There's long-term reduction in papillary function after RUUO shown in preliminary studies. This is associated with reduction in expression of differentiation markers AQP1, but not AQP2, in the two papillary epithelial cells, loop of Henle (LOH) and collecting duct (CD), respectively. We hypothesize there is a reduction in the repair of LOH but not CD, resulting from reduced regeneration of LOH but not CD epithelium after RUUO.

Methods: To evaluate CD and LOH independent of their differentiation states, we genetically labeled CD or LOH lineages in the renal papilla by crossing HoxB7 or Six2 Cre mice with a Cre-activated tdTomato fluorophore, respectively. Kidneys were collected at days 0, 3, and 7 for short-term and days 28 and 84 for long-term studies after RUUO. Kidney sections were stained with fluorescent AQP1 and AQP2 antibodies for long-term studies and Ki-67 antibodies, a marker of proliferative regeneration, for short-term studies. Digital images were quantified using QuPath.

Results: In long- term studies, the CDHox-B7 displayed no change in the papilla, and the LOH Six2 marker showed a decrease in the cells after RUUO compared with controls. In short-term studies, proliferation (Ki67) increased equally in HoxB7 and Six2 lineages after days 0 and 3 compared with healthy controls.

Conclusion: Our studies show that there is reduced long-term repair of LOH but not CD epithelium, but that is not associated with reduced regenerative repair of LOH. Future studies will explore other mechanisms of reduced long-term rapair of papillary LOH after RUUO, including analysis of differences in LOH and CD cell death at early time points.

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2. BNP and cGMP are Linked to Increased Variation in Transpulmonary Pressure in PH-HFpEF Patients

Presenter: Sophia Serra Tully, Northwestern University

Program: PAECER

Principle Investigator: Dr. Vineet Agrawal, M.D., Ph.D., Department of Medicine Division of Cardiology

Additional Project Authors: Elizabeth Kobeck, Department of Medicine Division of Cardiology

Abstract:

Background: The most common form of pulmonary hypertension is heart failure with preserved ejection fraction (PH-HFpEF) which is associated with increased mortality. Deficiencies in natriuretic peptide (NP) signaling potentially contribute to PH-HFpEF. This study investigated whether NP levels differ across the lung in patients with PH-HFpEF versus control. We hypothesized that patients with PH-HFpEF will have a greater transpulmonary gradient of NP levels than patients without PH or HFpEF.

Methods: Plasma was obtained from the pulmonary artery and capillary blood of patients undergoing catheterization for evaluation of PH. Levels of ANP, BNP, and cGMP were measured in plasma by ELISA. Significant differences were identified by the Mann-Whitney test.

Results: We recruited 11 patients with PH-HFpEF and 25 control patients. The median BNP level for PH-HFpEF patients vs. controls was 3900 pg/ml vs. 256 pg/ml ($p = 0.006$). The median transpulmonary gradient of BNP was also higher for PH-HFpEF patients vs. controls (1224 vs. 72 pg/ml, $p = 0.0001$). Transpulmonary cGMP was also higher for PH-HPpEF patients versus control (7.1 vs. -7.6 nM, $p = .02$). No difference was observed in the baseline or transpulmonary levels of ANP between PH-HFpEF and control.

Conclusions and Future Directions: Our findings show that levels and transpulmonary gradients of BNP and cGMP are higher in PH-HFpEF vs. controls. These findings support our hypothesis that the lung may contribute to decreased levels of BNP, which may affect the cGMP gradient across the lung. Future studies are needed to determine the mechanism by which BNP levels decrease across the lung.

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3. Association of HIV With Incident Venous Thromboembolism Among 143,461 Veterans

Presenter: Suzanne Alicia Navarro, Vanderbilt University

Program: PAECER

Principle Investigator: Aaron Aday, M.D., MSc, Department of Cardiovascular Medicine

Abstract:

Background: Prior data suggest people with human immunodeficiency virus (PWH) are at increased risk of venous thromboembolism (VTE). We hypothesized that this risk would be attenuated in a contemporary cohort with sustained viral suppression.

Methods: We analyzed data from the Veterans Aging Cohort Study, a longitudinal, observational, prospective study of PWH matched 1:2 with veterans without HIV. After excluding patients with prevalent VTE, we calculated VTE incidence rates and assessed the association between HIV infection and incident VTE using Cox proportional hazards models. We defined incident VTE using ICD-9/10 codes and adjusted models for demographics and VTE risk factors. Additional analyses explored the association between CD4+ T cell count or HIV viral load and incident VTE.

Results: Among 143,461 participants (30% PWH), there were 8,502 incident VTE events (33% among PWH). Rates of incident VTE per 1000 person-years were higher for PWH (6.41; 95% CI, 6.17-6.65) than those without HIV (5.09; 95% CI, 4.96-5.23). PWH were at increased risk of VTE (HRadj, 1.48; 95% CI, 1.41-1.56) compared to those without HIV. The risk was highest among those with time-updated CD4+ T cell counts <200 cells/mm3 (HRadj, 2.78; 95% CI, 2.52-3.06) or HIV viral loads >500 copies/mL (HRadj, 1.89; 95% CI, 1.74-2.05).

Conclusions: In a large, racially diverse cohort, HIV infection was associated with increased risk of incident VTE. This risk was greatest in those with low CD4+ T cell counts or elevated HIV viral loads. These findings may help risk stratify patients and potentially guide prophylactic interventions in the future.

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4. Role of IL-17R Signaling in the Stomach Epithelium During *H. Pylori* Infection

Presenter: Eseoghene Ivie Ogaga, Tennessee State University

Program: RISE UP

Principle Investigator: Holly Algood, Ph.D., Department of Medicine, Division of Infectious Diseases, Department of Veterans Affairs

Abstract:

Objective and Hypothesis: *Heliobacter pylori* is a pathogen that colonizes the stomach and drives gastritis, peptic ulcer disease, and gastric cancer. The T cell cytokine, Interleukin-17 (IL-17), plays an important role in the inflammatory response during *H. pylori* colonization of the gastric mucosa. This cytokine mediates protective innate immunity against pathogens by mobilizing neutrophils. The IL-17 receptor is expressed on epithelial cells, fibroblasts, and hematopoietic cells. Previous work suggests that a deficiency in IL-17R leads to increased chronic inflammation compared to control mice. Further, our lab has shown that IL-17R plays a significant role in activating antimicrobial components, including pIgR, which facilitates the transport of polymeric IgA across the epithelium. In this study, *Il17ra*$^{\Delta epi}$ and *Il17ra*$^{fl/fl}$ (control) mice were used to test the hypothesis that IL-17RA signaling in epithelial cells maintains barrier function and protects against hyperinflammation after *H. pylori* infection.

Approach: , *Il17ra*$^{\Delta epi}$ and *Il17ra*$^{fl/fl}$ (control) mice were infected with *H. pylori* and the impact of IL-17RA deficiency was investigated by assessing histological changes, gene expression changes and changes in IgA in the gastric wash.

Results: By one-month post-infection, gene expression analysis (Real-time RT-PCR) revealed decreased *Pigr*, *Cxcl1*, and *S100a8* expression in *Il17ra*$^{\Delta epi}$ mice compared to the control mice. Few differences in inflammation, pro-inflammatory markers, and IgA levels (ELISA) were observed. At three months-post-infection, despite a persistent reduction in *Pigr*, *Il17ra*$^{\Delta epi}$ mice showed increased IgA levels and pro-inflammatory markers (*Il21, Il17a*, and *Cd19*). Based on histological analysis, *Il17ra*$^{\Delta epi}$ mice often develop more lymphoid follicles and increased acute and chronic inflammation compared to control mice.

Conclusion: These data suggest that a deficiency of IL-17RA in epithelial cells is required to prevent chronic inflammation during infection and maintain barrier integrity but is not required to recruit PMNs.

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5. Diffusion MRI and Gene Expression in Brain Microstructure of Patients with Alzheimer's Disease

Presenter: Ellie Kowitz, Lipscomb University

Program: UCRIP

Principle Investigator: Derek Archer, Ph.D., Department of Neurology

Abstract: Diffusion MRI (dMRI) is an analytical imaging technique that utilizes magnetic fields to observe the movement of fluid in the brain to assess white matter tract and brain microstructure. When utilized longitudinally on individuals with Alzheimer's Disease (AD), dMRI can produce helpful data regarding the abnormal deterioration of the brain that accompanies AD. Coupling dMRI and genomic data has revealed significant correlations between the expression of certain genes and microstructural differences in AD patients. The research conducted for this study sought to identify and understand the most significantly correlated genes to AD microstructure in 3 regions: the caudate, dorsolateral prefrontal cortex (DLPFC), and posterior cingulate cortex (PCC). Statistical analysis in R was used to evaluate merged gene expression, dMRI, and covariate data to isolate key genes of interest based on the strength of their associations with brain microstructure in each of the chosen regions. Once these genes were distinguished, a literature review was performed to better understand and formulate hypotheses regarding how their functions might relate to AD. Conducting this literature review reaffirmed the complexity of AD and highlighted genes encoding for proteins with varying functions. Each region had unique genes correlated to their microstructure, supporting that there are many physiological processes at play in the Alzheimer's brain at the molecular level. Recognizing these significantly correlated genes and understanding their functions allows researchers to better understand the pathology of this life-altering disease and provides a foundation of knowledge to move forward in developing better preventative and treatment methods.

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6. Sam50 is Associated with Fragmentation and Alterations in Metabolism in Human and Mouse Myotubes

Presenter: Qiana Jianni Tanael Williams, Miles College

Program: PAECER

Principle Investigator: Antentor Hinton, Jr., Department of Molecular Physiology and Biophysics

Additional Project Authors: Heather Beasley, Andrea Marshall, Chia Vang, Zer Vue, Larry Vang, Department of Molecular Physiology and Biophysics

Abstract: Background: The Sorting and Assembly Machinery (SAM) Complex is responsible for assembling β-barrel proteins in the mitochondrial membrane. Comprising three subunits, Sam35, Sam37, and Sam50, the SAM complex connects the inner and outer mitochondrial membranes by interacting with the mitochondrial contact site and cristae organizing system (MICOS) complex. Sam50, in particular, stabilizes the mitochondrial intermembrane space bridging (MIB) complex, which is crucial for protein transport, respiratory chain complex assembly, and regulation of cristae integrity. While the role of Sam50 in mitochondrial structure and metabolism in skeletal muscle remains unclear, this study aims to investigate its impact.

Hypothesis: We hypothesized that the loss of Sam50 in myotubes increases mitochondrial fragmentation, increase autophagosome formation, and alters mitochondrial metabolism in skeletal muscle, thus, implicating the SAM complex as a modulator beyond only cristae integrity.

Methods: Serial block-face-scanning electron microscopy (SBF-SEM) and computer-assisted 3D renderings were employed to compare mitochondrial structure and networking in Sam50-deficient myotubes from mice and humans with wild-type (WT) myotubes. Mitochondrial metabolic phenotypes were assessed using Gas Chromatography-Mass Spectrometry-based metabolomics to explore differential changes in WT and Sam50-deficient myotubes.

Results: The results revealed increased mitochondrial fragmentation and autophagosome formation in Sam50-deficient myotubes compared to controls. Metabolomic analysis indicated elevated metabolism of propanoate and several amino acids, including ß-Alanine, phenylalanine, and tyrosine, along with increased amino acid and fatty acid metabolism in Sam50-deficient myotubes. Furthermore, impairment of oxidative capacity was observed upon Sam50 ablation in both murine and human myotubes, as measured with the XF24 Seahorse Analyzer.

Conclusion: Collectively, these findings support the critical role of Sam50 in establishing and maintaining mitochondrial integrity, cristae structure, and mitochondrial metabolism. By elucidating the impact of Sam50 deficiency, this study enhances our understanding of mitochondrial function in skeletal muscle.

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7. The Individual and Combined Association of Preoperative Sleep Disturbance and Depression on Disability and Pain after Lumbar Spine Surgery

Presenter: Joseph Girgis Helmy, Lipscomb University

Program: UCRIP

Principle Investigator: Kristin Archer, Ph.D., DPT, Department of Orthopaedic Surgery

Project Mentor: Rogelio Coronado Ph.D., DPT, Department of Orthopaedic Surgery

Abstract: Sleep disturbance (SD) and depression are risk factors for poor outcomes in patients with chronic pain. While depression has been examined in the context of lumbar spine surgery (LSS), less is known about the association between SD and postoperative spine outcomes. The objective of this study was to examine the individual and combined association between preoperative SD and depression with outcomes after LSS. National registry data from 706 patients undergoing LSS (mean age = 61.0 years, 37% female, 52% fusion) were examined. SD and depression were measured using PROMIS measures. Disability and back pain intensity were assessed preoperatively and at 12 months with the Oswestry Disability Index and Numeric Rating Scale, respectively. Patients were categorized as having no/mild vs moderate/severe SD and depression using PROMIS cutpoints. A 4-level variable for combined SD and depression status was created. The associations between SD, depression, and combined status with postoperative outcomes were examined in multivariable regressions. Preoperatively, 173 (25%) patients reported moderate/severe SD and 131 (19%) patients reported moderate/severe depression. When accounting for both factors, 135 (19%) patients reported moderate/severe SD without depression, 76 (11%) patients reported moderate/severe depression without SD, and 151 (21%) patients reported both moderate/severe SD and depression. In independent models, SD and depression were significantly associated with disability and back pain intensity (p < 0.05). However, only depression maintained a significant association when both factors were included in the same model. For the combined association, patients with moderate/severe SD and depression reported the highest 12-month disability and back pain intensity.

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8. 3D Reconstruction of Aged Brown Adipose Tissue Shows Cristae and Mitochondria Changes

Presenter: Jessica M. Bedenbaugh, Tennessee State University

Program: PAECER

Principle Investigator: Antentor Hinton, Jr., Department of Molecular Physiology and Biophysics

Abstract: Mitochondria are required for energy production and even give brown adipose tissue (BAT) its

characteristic color due to their high iron content and abundance. BAT is implicated in many critical cellular functions including thermogenesis. The physiological function and bioenergetic capacity of mitochondria are connected to the structure, folding, and organization of its inner-membrane cristae. During the aging process, mitochondrial dysfunction is observed, and the regulatory balance of mitochondrial dynamics is often disrupted, leading to increased mitochondrial fragmentation in aging cells.
We hypothesized that significant morphological changes in BAT mitochondria and cristae would be present with aging, with age-dependent losses emblematic of impaired ATP generation.
We developed a quantitative three-dimensional (3D) electron microscopy approach to map cristae network organization in mouse BAT to test this hypothesis. Using this methodology, we investigated the 3D morphology of mitochondrial cristae in adult (3-month) and aged (2-year) murine BAT tissue via serial block face-scanning electron microscopy (SBFSEM) and 3D reconstruction software for manual segmentation, analysis, and quantification.
Upon investigation, we found increases in mitochondrial volume, surface area, and complexity and decreased sphericity in aged BAT, alongside significant decreases in cristae volume, area, perimeter, and score. This suggests mitochondrial swelling, which can indicate cellular stress and altered environments, with mitochondria dynamically responding to these environments. Cristae became smaller with age indicating a reduced capacity for ATP generation.
Based on this structural study it is possible that age-related BAT alterations may arise more due to cristae changes than mitochondrial changes. While we are limited in looking at a male murine model, the data here still define the nature of the mitochondrial structure in murine BAT across aging. Future studies may further elucidate how these structural changes affect ATP generation and the thermogenic properties of BAT.

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9. Analysis of Phenotypes in UTI Related *E.coli* Knockouts

Presenter: Briana Harness, Western Kentucky University

Program: MSTP Summer Research Program

Principle Investigator: Megan Behringer, Ph.D., Department of Biological Sciences

Project Mentor: Owen Hale Ph.D. student, Department of Biological Sciences

Additional Project Authors: Michelle Yin

Abstract: Approximately half of all women will experience a urinary tract infection (UTI) within their lifetime, many of whom will experience recurrent infections. *Lactobacillus* performs a protective role within the urinary tract by preventing opportunistic pathogens such as *E.coli*, from colonizing the bladder through modulation of the host and the secretion of antimicrobial agents. Loss of function *E. coli* mutants that are resistant to inhibition by *Lactobacillus* were identified by a genetic screen. To better understand the relevance of these mutations to *E. coli* physiology in the bladder, we evaluated the growth of mutants under sixteen stressors to simulate the adverse conditions that *E. coli* must overcome when colonizing the bladder and causing a UTI. It was found that mutants ΔcpxA, ΔompR, and ΔenvZ are likely relevant to the bladder microbiome due to their ability to grow under various pH, hydrogen peroxide, and antibiotic stressors. Of the mutants, ΔenvZ performed most comparably to the wild type, even exceeding the wild type in some tests. This presses upon the importance of evolutionary and ecologically rooted investigations of the urinary tract microbiota. By understanding the genetic and phenotypical relationships between *Lactobacillus* and evading *E.coli,* a better understanding of the

cause of urinary tract infections can be established and furthermore, a more personalized approach to the treatment of UTIs can be developed.

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10. Mitochondrial Morphology Regulates Proximal Tubule Cell Differentiation Status

Presenter: Freddiemae Thompson, University of Arkansas at Pine Bluff

Program: Aspirnaut

Principle Investigator: Craig Brooks, Ph.D., Department of Medicine, Division of Nephrology and Hypertension

Project Mentor: Sho Sugahara, M.D., Ph.D., Department of Medicine, Division of Nephrology and Hypertension

Abstract: Acute kidney injury (AKI) is defined by the sudden reduction in function. Although often reversible, AKI can transition to chronic kidney disease (CKD), a condition that is characterized by progressive kidney damage and affects approximately 10% of the world's population. Prolonged maladaptive dedifferentiation of kidney proximal tubular cells (PTCs) has been regarded as a cause of AKI to CKD transition. PTCs are highly metabolically active and have an abundance of mitochondria to satisfy their requirements. We previously found that intracellular mitochondria are fragmented in maladaptive dedifferentiated PTCs. Here we show that correction of fragmented mitochondria through deletion of dynamin-related protein 1 (Drp1), reverses the maladaptive dedifferentiation of PTCs and prevents AKI to CKD transition in mice. Specifically, kidneys of control mice showed severe fibrosis, PTC dedifferentiation, mitochondrial fragmentation, and senescence 6 weeks after administration of the nephrotoxic drugs aristolochic acid and cisplatin. By contrast, doxycycline-induced knockout of Drp1 in PTCs 2 weeks following injury preserved mitochondrial fragmentation and protected mice from kidney fibrosis. Our findings suggest that interventions that preserve mitochondria morphology may aid in cell differentiation and CKD alleviation.

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11. Inflammation Alters the Elastic Fiber Assembly in the Postnatal Mouse Lung

Presenter: Bailey Groff, Elizabethtown College

Program: Aspirnaut

Principle Investigator: John Benjamin, M.D., M.P.H., Department of Pediatrics

Project Mentor: Reit van der Meer, Department of Pediatrics

Abstract: The extracellular matrix (ECM) provides structural support and mechanical strength to the developing lung. Elastic fibers are critical components of the ECM and enable elastic recoil of the lung during breathing. These fibers are cord-like structures made from various assembly component proteins, including elastin, fibulin-5, and loxl1, which are thought to assemble during the saccular stage of lung development (embryonic day 18 to postnatal day 5 in mice; 24 – 32 weeks gestation in humans). The inability to assemble elastic fibers during this developmental stage as a result of noxious insults such as inflammation could have long-term consequences for the lung. In two different models of saccular stage lung inflammation, we used Hart staining to demonstrate the fragmentation of elastic fibers, resulting in both short- and long-term abnormalities in the elastic fiber architecture. Changes were also observed in the mRNA expression of elastin assembly components, elastin, fibulin-5 and Loxl1, by both real-time quantitative PCR (qRT-PCR) and RNA in situ hybridization (RNAscope) in these models. Restoring

homeostasis in elastic fiber organization by maintaining expression of elastin assembly components may be important for preserving long-term lung structure and function.

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12. Exploring Digital Tools to Ease Patient Uncertainty in the Emergency Department

Presenter: Matthew Ahlers, Duke University and Sherwin Samuel Newton, Baylor University

Program: UCRIP

Principle Investigator: Ryan Buckley, M.D., Department of Medicine

Additional Project Authors: Sherwin Newton

Abstract: Emergency Department (ED) patients experience many unknowns due to the complex and hectic nature of ED workflows. Often, patients encounter anxiety that stems from a natural instinct to fear uncertainty. We hypothesize that implementing a multimedia guide through the care systems of the ED will help reduce patient anxiety. In turn, patient satisfaction and clinical outcomes will be improved, as this decreased anxiety will allow for improved comprehension of the medical plan. We employed a human-centered design approach. This team worked to develop the above hypothesis and identify underlying drivers of patient anxiety by shadowing and interviewing multiple stakeholders from the VUMC ED. Over the course of 8 weeks, 12 doctors and nurses were interviewed. Their insights affirmed that patient confusion and anxiety most commonly stems from the workflow of the ED, boarding in hallway beds, interpreting ID badges, distinguishing roles as people enter their room, and misidentifying care providers due to implicit bias towards black and/or female physicians. Based on these results, we concluded that a map-based app with integrated videos is needed to orient and educate patients. Unlike past patient education videos, this product needs to be intuitive, just-in-time, and visually appealing so that patients actually use it. 70,000 adults and 50,000 children use the VUMC ED per year. If successfully supported, this QI project can greatly alleviate patient anxiety which can help lead to improved clinical outcomes and patient satisfaction.

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13. Super-Structural Organization of Collagen IV Scaffold in Kidneys

Presenter: Jennifer Diaz Sales, Berea College

Program: Aspirnaut

Principle Investigator: Sergei Boudko, Ph.D., Division of Nephrology and Hypertension, Department of Medicine

Abstract: Collagen IV scaffolds are found in all the basement membranes of all species of the animal kingdom. In humans, it can be found in many tissues including the kidneys. Collagen IV has six genes that encode six alpha chains, and three alpha monomers assemble to form α112, α345, and α556 protomers in the endoplasmic reticulum. Once secreted outside, the protomers get exposed to the high concentration of chloride in the extracellular space, which leads to the head-to-head association of two protomers (NC1 hexamer) followed by the tail-to-tail association of four protomers (7S domain) to build the scaffold. Abnormalities in the formation of protomers, and also the NC1 hexamer of α345 are associated with Alport syndrome. Currently, no cure for Alport syndrome is available. The molecular mechanism of this syndrome is unclear which complicates its prevention and therapy. Despite extensive study of the structure of α112, very little is known about the super-structural organization of α 345 and α556. Specifically, it is unknown whether α345 protomers can associate head-to-head with any other protomer or whether 7S-like structures exist that involve α345 and α556 protomers. We determined the

amount of Collagen IV in cow kidneys and attempted the separation of different NC1 hexamer isoforms using ion exchange chromatography. We also confirmed glycosylation of the 7S domain and found glycosylation of NC1, which was not reported before. A better understanding of the super-structural organization of α345 protomers in the scaffold will assist in the development of a protein replacement therapy for Alport patients.

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14. Development of a Western Protocol for Detection of TNC in Thyroid Cancer Cells

Presenter: Ryan Xavier, Sewanee: The University of the South

Program: V-SURE

Principle Investigator: Vivian Weiss, M.D., Ph.D., Department of Pathology, Microbiology, and Immunology

Project Mentor: Heather Hartmann, Department of Pathology, Microbiology, and Immunology

Additional Project Authors: Matthew Loberg, Department of Cancer Biology; Sara Kassel, Department of Cell and Developmental Biology; Ethan Lee MD PhD, Department of Cell and Developmental Biology

Abstract: Thyroid cancer is predicted to be the fourth leading cause of cancer diagnosis by 2030. Anaplastic thyroid cancer (ATC) is the most lethal thyroid cancer, and this increased aggression has been attributed to alterations in the Wnt signaling pathway. In fact, Wnt signaling is a known prover of carcinogenesis in many tissues. In both ATC patient tumors and ATC tumor cells, Tenascin-C (TNC) has been found to be upregulated and along the leading edge of the tumor. The role of TNC upregulation in ATC tumor cells is unknown. We are interested in exploring the relationship between TNC and Wnt signaling. We hypothesize that TNC binds Wnt and accumulates the ligand for more accessible receptor binding. Using Western blot techniques, we have analyzed the presence of TNC protein in both cell lysate and supernatant to understand more about the role of TNC in ATC. Because TNC is an extracellular matrix protein, we are interested in both its presence within cells and in secreted proteins found in cell supernatant. We found that TNC is present in both our control and transient transfection; however, there is not a difference in TNC protein expression. A lack of a distinct difference between control and transient transfected cells may indicate issues with the transfection process including collecting at the wrong time point. Future directions include repeating Westerns to collect more consistent data and repeating transfections with differing collection time points. Understanding how TNC interacts with the Wnt signalosome could lead to novel targeted cancer therapeutics.

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15. Studying the Mechanisms of Transcriptional Synergy Between STAT1 and NFκB

Presenter: Maci Fulton, Florida Agricultural and Mechanical University

Program: PAECER

Principle Investigator: Jonathon Brown, M.D., Department of Cardiovascular Medicine

Additional Project Authors: Emily Carson, Lindsay Davison, Dennis Buehler, Phuong Pham, Tuerdi Subati, Ronan Bracken, Jonathon Brown, Department of Cardiovascular Medicine

Abstract: Background: Transcription factors p65 and STAT1 regulate proinflammatory gene programs. When both are activated, proinflammatory gene expression is synergistically induced, which can significantly increase the level of inflammation at sites of injury or infection. Our group has identified a subset of genes induced by interferon gamma (IFNg)-STAT1 and tumor necrosis alpha (TNFa)-p65 in

endothelial cells (ECs). A major question remains how STAT1 and p65 are recruited to genes and interact at the level of enhancer DNA. Notably, a major limitation for evaluating their function is that existing antibodies are not of sufficient quality to landscape their occupancy at DNA.

Objective: To engineer EC lines that express epitope-tagged forms of STAT1 and p65, thereby enabling genomic occupancy studies using ChIP-sequencing and CUT&RUN.

Methods/Results: Using gateway cloning, 3xFLAG tags were inserted at the N-terminus of p65 and STAT1 cDNAs. The expression of these constructs was verified using transient transfection and immunoblotting with an anti-Flag antibody. Stable cell lines that will express 3x-FLAG-p65 or 3xFLAG-STAT1 in endothelial cells are now in development, in which the endogenous p65 and STAT1 have already been knocked out by CRISPR/Cas9. HEK 293T/17 cells were transfected with plasmid constructs for lentivirus production and virus harvested after 48 hours. ECs were infected with virus and selected over 10 days with blasticidin antibiotics.

Ongoing Work: p65 and STAT1 genome occupancy studies will be undertaken in ECs stimulated with IFNg and TNFa. The ultimate goal is to gain insight into the mechanisms of STAT1/p65 transcriptional synergy in ECs.

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16. Overexpression of PMP22 increases autophagy in rat Schwann cells

Presenter: Gagan Phuyal, Berea College

Program: Aspirnaut

Principle Investigator: Bruce Carter, Ph.D., Department of Biochemistry, Vanderbilt Brain Institute

Project Mentor: Pramod Gowda, Ph.D., Department of Biochemistry, Vanderbilt Brain Institute

Abstract: Charcot-Marie-Tooth disease type 1A (CMT1A) is a common inherited peripheral neuropathy that results from the duplication of PMP22, which encodes peripheral myelin protein 22 (PMP22). Affected Schwann cells, which are responsible for producing and maintaining the myelin sheath around axons, consequently overexpress PMP22, which forms aggregates within the cytosol and potentially compromises cell function and viability. This, in turn, leads to demyelination, axonal degeneration, progressive muscle weakness, and sensory loss. Autophagy is a cellular mechanism that degrades unwanted or damaged components; however, its potential role in clearing PMP22 aggregates remains unclear and inadequately investigated. Here, we show that autophagy is increased in Schwann cells that overexpress PMP22. Specifically, we demonstrate that doxycycline-induced overexpression of PMP22 in cultured rat Schwann cells triggers an increase in the autophagy marker LC3B, as determined by Western blotting. This finding strongly suggests that the Schwann cells use autophagy as a mechanism to mitigate the aggregation of PMP22. Our findings suggest that induction of autophagy may represent a potential therapeutic target for individuals with CMT1A, as a means to enhance the clearance of PMP22 aggregates, and thereby alleviate the pathological consequences arising from PMP22 overexpression.

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17. Dopamine release to conditioned aversive stimuli is time and learner-type dependent

Presenter: **Dopamine release to conditioned aversive stimuli is time and learner-type dependent**

Presenter: Jaela G. Melton, North Carolina Agricultural and Technical State University

Program: MSTP Summer Research Program

Principle Investigator: Erin Calipari, Ph.D., Department of Pharmacology

Project Mentor: Stephanie Cajigas, Vanderbilt Brain Institute, Vanderbilt School of Medicine

Abstract: To understand how and why behaviors occur, and critically how they are dysregulated in disease (ex. substance use disorders (SUD), we need to know what environmental stimuli are important and drive behavioral responses. The SUD field focuses on how drugs drive future behavior, how associations are formed between drugs and stimuli, and how learning becomes maladaptive. However, to investigate how learning becomes dysregulated in SUD we first need an understanding of the neural circuits underlying associative learning. To do so, we utilized an optical sensor that binds dopamine, dLight, with in-vivo fiber photometry in the nucleus accumbens core to monitor real-time dopamine responses during negative reinforcement. Each trial began with a 15-second tone and lever presentation, after which mice received a series of 5 shocks, followed by a house light (safety cue) and a variable inter-trial interval. Here, a lever can be pressed to end the trial any time between the tone start and the last shock to prevent or stop future shocks. We found mice that acquire negative reinforcement (learners) display less freezing and more lever pressing over time, while those that do not learn the paradigm (non-learners) have no change in either. Additionally, to the aversive cue, learners have an increase in dopamine release over time, while non-learners exhibit a decrease. These data demonstrate behavioral changes dependent on learner types and changes in dopamine signaling that may underly how a subject navigates their environment and perceives the availability of behavioral outcomes (ability to escape shocks vs perceived inescapability).

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18. Investigating the p75 Neurotrophin Receptor Signaling Pathway in Schwann Cells

Presenter: Nawshin Maleeha, Macaulay Honors College at CUNY Hunter College

Program: BP-ENDURE

Principle Investigator: Bruce Carter, Ph.D., Department of Biochemistry

Project Mentor: Vishwanath Prabhu, Ph.D., Department of Biochemistry

Abstract: The regulation of lipid metabolism in Schwann cells is critical to proper myelin formation and sensory neuron survival in the peripheral nervous system. Previous research from the Carter Lab identified the importance of p75 neurotrophin receptor (NTR) expression for the regulation of lipid metabolism in Schwann cells, finding that Schwann cell-specific deletion of p75NTR resulted in disruptions in cholesterol biosynthesis and 30% loss of dorsal root ganglion neurons. Despite this discovery of p75NTR's role in Schwann cells, the signaling pathway by which p75NTR activates lipid metabolism genes is not fully understood. Previous studies in hepatocytes reported that neurotrophins like nerve growth factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF) activate sterol regulator-element-binding protein-2 (SREBP2), involved in regulation of lipids and cholesterol-associated genes, through p75NTR activation of p38 MAP kinase and caspases. Therefore, we investigated SREBP2 activation by p75NTR via a similar downstream signaling cascade within Schwann cells. We treated wild-

type rat Schwann cells with BDNF for 1 hour, 3 hours, 6 hours, and overnight to activate the p75NTR response. At each time point, we quantified the protein expression level for various proteins hypothesized to be involved in the p75NTR signaling pathway, such as p38 MAPK, caspase-2, and SREBP-2. If involved in the pathway, we predict that BDNF treatment will increase the active form of these proteins: phosphorylated p38 MAPK, cleaved caspase-2, and cleaved SREBP-2. Determination of the p75NTR pathway in Schwann cells will be fundamental to understanding and intervening in various peripheral neuropathies that relate to Schwann cell lipid dysfunction.

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19. NAPE-Hydrolyzing Phospholipase D as a Potential Target for Therapeutic Interventions against Cardiometabolic Disease

Presenter: Stetler Tanner, Brigham Young University

Program: Independent Research Intern

Principle Investigator: Sean Davies, Ph.D., Department of Pharmacology

Additional Project Authors: Abdul-musawwir Alli-oluwafuyi, Zahra Mashhadi, Department of Pharmacology

Abstract: Cardiometabolic diseases (CMD), including atherosclerotic cardiovascular disease, affect a quarter of the general US population, making them a focus for therapeutic approaches. Atherogenesis is closely linked to inflammation, and macrophages play an important role in inciting and resolving inflammation. Resolution of inflammation requires efficient efferocytosis. Impaired efferocytosis results in excessive lipid buildup and expansion of necrotic cores within the arteries, resulting in vulnerable atherosclerotic plaques prone to rupture. NAPE-hydrolyzing phospholipase D (NAPE-PLD) is a zinc metallohydrolase that produces N-acyl-ethanolamides, such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA). Treatment with bacteria engineered to increase production of PEA and OEA has been shown to provide protection against CMD. NAPE-PLD was also shown to be downregulated in atherosclerotic plaques. Recent studies in the Davies lab showed that activating NAPE-PLD enhanced efferocytosis, while inhibiting it produced the opposite effect. Bulk RNA-sequencing of bone marrow-derived macrophages (BMDM) from wild-type and Nape-pld knock-out (KO) mice revealed several genes that were downregulated in the KO BMDM. Using qPCR, I verified the downregulation of a number of these genes in KO BMDM including Acp1, Odc1, Hmox1, and Lpin1. I also found that treatment of KO BMDM with PEA restores gene expression of Acp1, Hmox1, and Lpin1. To determine if Nape-pld forms complexes with these or other proteins, I transfected cells with a His-tagged Nape-pld, performed crosslinking to stabilize protein complexes, and am now analyzing them by SDS-PAGE. By further elucidating the role of NAPE-PLD in macrophages' functions, including efferocytosis, we may be able to develop novel therapeutics for CMD.

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20. The Development of Face Processing Related Brain Structures in Individuals With Varying Likelihoods of Autism

Presenter: Maylyn Mei, Hunter College

Program: BP-ENDURE

Principle Investigator: Carissa Cascio, Ph.D., Department of Psychiatry and Behavioral Sciences

Project Mentor: Alisa Zoltowski,

Abstract: The fusiform gyrus (FG) is responsible for higher visual perception, specifically the ability to process faces, objects, and written words. Within the FG, the fusiform face area (FFA) recognizes and perceives faces, with face recognition and perception accuracy differing between neurotypical and autistic individuals. The mid-fusiform sulcus (MFS) divides the FG into medial and lateral sections, identifying functional regions in the FG, one of which is the FFA. Studies have shown that neurotypical individuals with better facial recognition typically have thinner FFAs while in autistic individuals, cortical thickness CT predicts recognition ability for both face and non-face objects, demonstrating less specificity for faces. Using the MFS to predict the location of FFA, we will calculate the FFA's CT and sulcal depth (SD). As facial recognition is one of the earliest developmental hallmarks, we will be using structural MRI scans of six, twelve, and twenty-four-month-old infants with differing likelihoods of autism, analyzing when the MFS develops in relation to when they learn to process and recognize faces. We will also be able to identify differences in CT and SD of infants with a low and high likelihood of autism. Starting with the MFS identification, analysts will manually examine T1-weighted images from 503 infants and verify MFS presence. After identification, analysts will trace the boundaries of the fusiform and grey matter & white matter and grey matter boundaries. Using the tracings and Matlab coding, we will be able to accurately determine the average distance between the boundaries, determining the CT.

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21. The Role of Epithelial Plasticity in Pancreatic Tumorigenesis

Presenter: JoAnna Dennis, Alabama Agricultural and Mechanical University

Program: Aspirnaut

Principle Investigator: Kathleen DelGiorno, Ph.D., Department of Cell and Developmental Biology

Project Mentor: Sergey Ivanov, Department of Cell and Developmental Biology

Abstract: Pancreatic ductal adenocarcinoma (PDAC) represents an extremely aggressive malignancy characterized by poor prognosis and a low overall survival rate. These dire statistics can be attributed to the fact that PDAC evades detection until it has progressed to metastatic, incurable stages. Acinar to ductal metaplasia (ADM) is an early event in pancreatic injury where digestive enzyme-producing acinar cells become ductal-like. This process is considered to facilitate healing following injury, but also serves as a potential precursor to PDAC. We have previously shown that ADM results in the formation of a heterogeneous population of cells, including gastric-like SPDEF+ and progenitor-like osteopontin-positive populations; however, the role of these cell populations in pancreatic tumorigenesis is unknown. Here, we demonstrate that osteopontin regulates epithelial plasticity and the formation of specific cell types. Histological analyses of pancreata from osteopontin-knockout mice that express oncogenic KrasG12D, the main driver of PDAC, revealed a decrease in epithelial plasticity and the formation of tuft and enteroendocrine cells, coincident with the production of benign, cyst-like lesions. The generation of PDAC cell lines with CRISPR-induced knockout of SPDEF will provide further insights into the functional roles of ADM populations in pancreatic tumorigenesis. These studies will reveal new roles for epithelial plasticity and potential pathways that may be targeted to inhibit PDAC formation.

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22. Identifying *Staphylococcus aureus* Agr-Regulated Toxins Enhanced by *Candida albicans* in Co-culture

Presenter: Isabelle Cruz Hill, Howard University

Program: PAECER

Principle Investigator: Jim Cassat, M.D., Ph.D., Department of Pediatric Infectious Disease

Project Mentor: Kara Eichelberger Ph.D., Department of Pediatric Infectious Disease

Abstract: Previous research shows that the fungus *Candida albicans* activates the *Staphylococcus aureus* accessory gene regulator (agr) quorum sensing system occurs during in vitro co-culture, and this contributes to enhanced mortality during *C. albicans-S. aureus* co-infection. The agr system regulates expression of almost all *S. aureus* toxins. Our group previously showed that *C. albicans* also enhances *S. aureus* Agr-independent cytotoxicity via a mechanism requiring Panton Valentine Leukocidin (PVL). The main goal of this project is to identify Agr-regulated toxins that contribute to human cell cytotoxicity in combination with PVL following *C. albicans* co-culture. We hypothesize that the Agr-regulated toxins psmα and α-toxin (hla) are the main toxins required. To test our hypothesis, we first used phage to transduce Δpsmα and Δhla constructs into Δpvl. Successful creation of Δpsmα/Δpvl and Δhla/Δpvl mutants was confirmed with PCR. We next sought to test cytotoxicity of our mutants towards human cells. First we optimized testing culture supernatant cytotoxicity on human A-549 cells and determined that 40% and 60% supernatant concentrations work best. We next collected supernatants from Δpsm, Δagr, Δpsm/pvl, and Δagr/Δpvl cultures grown with and without *C. albcians*. We expect that if psmα is important for Agr-mediated cytotoxicity towards human cells following *C. albicans* co-culture, then the Δpsm/Δpvl strain would be less cytotoxic, similar to the ΔagrΔpvl strain in co-culture. We found that the Δpsm/Δpvl strain still has enhanced cytotoxicity towards A-549 cells, but we also observed that the Δagr/Δpvl mutant also had enhanced cytotoxicity following co-culture. To confirm these unexpected results, we tested our Δpsm/pvl supernatants on human monocytes. We observed similar cytotoxicity results as with our A-549 cytotoxicity assay. Because our results were not as expected, we would repeat these experiments with new supernatant preparations. Overall, we were able to successfully create double-mutant strains Δpsmα/Δpvl and Δhla/Δpvl mutants and began testing cytotoxicity of these strains.

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23. Optimization of layer-by-layer Coating of SiRNA for Balloon Angioplasty

Presenter: Adrian Aligwekwe, North Carolina State University

Program: MSTP Summer Research Program

Principle Investigator: Craig Duvall, Ph.D., Biomedical Engineering

Project Mentor: William Tierney B.S., Biomedical Engineering

Abstract: A common issue associated with failure in vascular procedures is the formation of intimal hyperplasia (IH) characterized by an abnormal accumulation of vascular smooth muscle cells (VSMCs) in the intimal layer of blood vessels. This is caused by a phenotype switch derived from the p38 MAPKAP2 protein (MK2) inflammatory pathway stimulated by the mechanical stress from these procedures, leading to the occlusion of arterial walls. Previous studies have shown that inhibition of MK2 may limit the accumulation of smooth muscle cells. RNA interference (RNAi) using Short Interfering RNA (siRNA) can be used to block the translation of MK2 by binding to messenger RNA sequences that code for the protein. We aim to develop a delivery mechanism for siRNA via balloon angioplasty that pairs the

negative charge of nucleic acids with positively charged biocompatible polymers to layer a therapeutic dose of 1-2 nanomoles of siRNA on the balloon's surface. The current formulation utilizes polyurethane balloons that have been plasma treated and then functionalized with (3-aminopropyl) triethoxysilane to provide an initial cationic layer for siRNA molecules to bind. The following layers are added using positively charged polylysine, polyethyleneimine, and poly-dimethyaminoethylmethacrylate (PDMAEMA) to test the effects of charge density on the electrostatic complexation onto the balloon. Results have shown that using PDMAEMA we can load about 1 nanomole of siRNA using 8 layers, and we have successfully achieved delivery of siRNA to explanted rat aortas ex vivo. Future studies will test cell infiltration of siRNA molecules using flow cytometry.

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24. Effects of S100 Proteins on *E. coli* Biofilm Dynamics

Presenter: Melumo Togashi, Vanderbilt University

Program: Independent Research Intern

Principle Investigator: Walter J. Chazin, Ph.D., Department of Biochemistry and Chemistry

Project Mentors: Kyle T. Enriquez, M.Sc., Vanderbilt University Medical Scientist Training Program; Y. Randika Perera, Ph.D. Department of Biochemistry and Chemistry

Additional Project Authors: Tae Akizuki, Department of Biochemistry and Chemistry

Abstract: Biofilms are multi-cellular, three-dimensional bacterial communities anchored to a surface. *Escherichia coli* form biofilms that consist of a bacterial colony embedded in a matrix of extracellular polymeric substances. This protective matrix shields the microbes from adverse environmental conditions and the host immune system, leading to infection. To perturb these infections, a host employs "nutritional immunity," withholding vital nutrients like transition metals essential for pathogen survival. As part of nutritional immunity, hosts release S100 proteins such as S100A8/S100A9 (calprotectin, CP) and S100A7, EF-hand calcium-binding proteins that contribute to zinc-mediated nutritional immunity by sequestering essential metals.

Sequestration of multiple versus single metals is known to have differing impacts on biofilm kinetics. To monitor biofilm dynamics, Temporal Modeling of the Biofilm Life Cycle (TMBL) assays were performed, collecting data every 12 hours across 4 days. Standard growth curves and dose-dependent TMBL assays were used to evaluate metal sequestration by CP and S100A7 on planktonic and biofilm communities. The effect of Poly-n-acetyl glucosamine (PNAG), a polysaccharide that promotes biofilm formation, was examined to determine if S100A7 or CP perturb biofilm growth in the presence of this adherence promoter. CP and S100A7 have dose- and PNAG-dependent effects on the biofilm lifecycle. Overall, compared to CP, S100A7 has less deleterious effects on biofilm growth kinetics, which we attribute to the differences in the structure and Zn binding affinities of the two proteins. Future investigations will seek to determine what features of S100A7 and CP explain their differences in observed phenotypes.

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25. The Expression and Purification of Monoclonal Antibodies Using Expi293F™ Cells to Evaluate Viral Peptide Antigen Specificity

Presenter: Nadine Abazie, Howard University

Program: Aspirnaut

Principle Investigator: Ivelin Georgiev, Ph.D., Department of Pathology, Microbiology, and Immunology

Project Mentor: Gwen Jordaan, M.S., Department of Pathology, Microbiology, and Immunology

Abstract: Monoclonal antibodies can be used in many medical applications, including the treatment of bacterial and viral infections, by recognizing and binding to specific bacterial or viral epitopes. In this project, we expressed and purified four monoclonal antibodies (1-11, 1-14, 2-23, and 4-41) discovered in LIBRA-seq (Linking B cell Receptor to Antigen specificity through Sequencing), an application that allows for a high throughput screening method of multiple antigens. The antigens we focused on were five biotinylated antigens selected from a virome peptide library of known human viruses: Flu epitope, EBV epitope, Herpesvirus 5, Herpesvirus 6B, and Herpesvirus 4. We transformed DH5α competent cells with a plasmid containing either heavy chain (HC) or light chain (LC) DNA. The isolated DNA was then transfected into Expi293F™ cells. Expressed antibodies were purified by Protein A purification. The antibodies were evaluated by SDS-PAGE and ELISA. Our results showed the highest specificity binding for antibody 1-11 to the flu epitope; the 4-41 antibody showed high specificity binding for herpesvirus 6B; the 2-23 antibody showed moderately high specificity binding to the EBV epitope; the 1-14 antibody showed no binding to all the antigens; and none of the antibodies showed binding to the herpesvirus 5. Future analyses will involve a neutralization assay to determine each antibody's ability to neutralize a target antigen. In addition, an epitope mapping will reveal which amino acids on the antigen surface are engaged during the antibody binding.

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26. The Effect of Circadian Rhythms on the Number, Motility, and Speed of Macrophages in Zebrafish.

Presenter: Sarah Livingston, Sewanee: The University of the South

Program: V-SURE

Principle Investigator: Wenbiao Chen, Ph.D., Department of Molecular Physiology & Biophysics

Project Mentor: Brittney Covington, Molecular Physiology and Biophysics

Abstract: Type 2 diabetes has increased substantially in the United States due to obesity. Too much fat in the stomach and around the organs leads to insulin resistance, a stressor that results in beta cell loss. How and when beta cell death occurs is unknown, however, recent studies show that circadian rhythms may be connected to the beta cell function. More specifically, circadian rhythms may influence the motility of innate immune cells to wound sites in different mammalian models, and this timing could affect aspects of the beta cell function. Using a zebrafish model, we examine whether there is any change in their macrophage motility and speed to a wound site during different phases of a 24-hour circadian rhythm cycle. Six days post-fertilization, zebrafish with fluorescent-labeled macrophages are imaged using a confocal fluorescent microscope and studied throughout the day at four-hour intervals. Preliminary results show that as time passes during a 24-hour period, the number of macrophages decreases. This provides supporting evidence that more macrophages are present during the inactive phase in zebrafish. We are performing additional tests that involve disrupting the circadian rhythm in order to examine its impact and results are forthcoming. Further experiments could explore the motility and speed of other innate immune cells in zebrafish such as neutrophils.

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27. Pregnancy Reduces Renal Injury in Aged GPER1 Knockout Mice.

Presenter: Hailey Mackenzie Mullins, Spelman College

Program: PAECER

Principle Investigator: Eman Gohar, Ph.D., Department of Nephrology

Additional Project Authors: Ravneet Singh, Rawan N Almultaq, Juliet Umunna, Victoria Nasci, Department of Nephrology

Abstract: Estrogen, a crucial sex hormone, plays a significant role in kidney development and regulation. The incidence of kidney disease in women tends to increase after menopause, which is associated with decreased estrogen levels. G protein-coupled estrogen receptor 1, GPER1, is a membrane-associated estrogen receptor that is expressed in the kidney. Evidence implicates a protective role for pregnancy on the long-term cardiovascular health. I hypothesize that pregnancy mitigates renal injury in aged GPER1 knockout (KO) mice.

We utilized young (3 months old) and aged (16-20 months old) GPER1 wildtype (WT) and KO female mice. Aged mice were assigned into virgin groups or previously-pregnant groups. Urine samples and kidneys were harvested. Bradford assay was performed to determine urinary protein concentration as an indicator of renal injury. After kidney harvesting, the TUNEL assay, which identifies apoptotic cells, was performed. The number of apoptotic cells was counted to determine the extent of renal apoptosis.

Regardless of genotype, aged mice elicited greater proteinuria than young mice. GPER1 deletion increased proteinuria in aged mice. Interestingly, pregnancy reduced proteinuria in aged KO mice. GPER1 deletion did not impact apoptosis in young mice. However, aged virgin KO mice had greater apoptosis compared to age-matched virgin WT mice. TUNEL assay revealed that pregnancy resulted in lower apoptosis in aged KO mice. In summary, GPER1 deletion promotes proteinuria and renal apoptosis in aged mice and this effect is eliminated by previous pregnancy. This suggests a potential crosstalk between GPER1 signaling and pregnancy in preserving the long-term renal health in females.

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28. Spatial Analysis of Human Choroid Plexus Pathology in Dementia

Presenter: Emily Patmore, Butler University

Program: Aspirnaut

Principle Investigator: Neil Dani, Ph.D., Department of Cell and Developmental Biology

Project Mentor: Angela Wang, Department of Cell and Developmental Biology

Abstract: The choroid plexus is widely known for its role in producing cerebrospinal fluid (CSF) and is located within the ventricular system of the brain. Cells of the choroid plexus generate factors that support brain health and clear waste. CSF biomarker analyses have identified causative (e.g., β-amyloid) and neuroinflammatory factors for the diagnosis of neurodegenerative disease. However, the relationship between the choroid plexus, CSF, and impaired cognitive dysfunction in neurodegeneration is poorly understood. Recent single cell transcriptomic analyses of the choroid plexus have revealed a striking diversity of constituent cell types, which include endothelial and immune cell types of lymphoid and myeloid lineages. Using innovative approaches to investigate cellular and molecular features across the whole lateral ventricle choroid plexus (LVChP), here we demonstrate spatial associations between macrophages and concretions within the choroid plexus of patients with dementia and in aged tissue. Preliminary analyses using hematoxylin and eosin in 8 donor cases, including tissues from patients with

Alzheimer's disease, Frontotemporal dementia, Amyotrophic lateral sclerosis (ALS), and healthy aging, identifies enriched concretions in the posterior aspect of the tissue along vascular beds in all 8 samples. Immunohistochemistry reveals macrophages (Iba1+) distributed across the tissue and congregations around specific subtypes of concretions. Macrophage proximity suggests previously unappreciated immune responses to these putative immature structures, which may facilitate their maturation in several types of dementia. Our study sets the stage for further profiling of neuroinflammation in the choroid plexus.

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29. Developing of an Open-source Radiomics-based Risk Prediction Model for Indeterminate Pulmonary Nodules

Presenter: Ian Johnson, Colorado College

Program: UCRIP

Principle Investigator: Eric Grogan, M.D., MPH, Department of Thoracic Surgery

Project Mentor: David Xiao, M.D., Department of General Surgery

Abstract: Lung cancer claims the most lives of any malignancy worldwide, with 238,340 new cases and 127,070 annual deaths in the US alone. Recent lung cancer screening trials such as the NLST (National Lung Screening Trial) and the NELSON (Dutch-Belgian Randomized Lung Cancer Screening Trial) have demonstrated that regular screening of high-risk individuals leads to improved mortality. However, improvements in screening have led to several challenges, including a significant increase in the detection of indeterminate pulmonary nodules (IPNs). IPNs require further assessment either through longitudinal monitoring or diagnostic procedures. These procedures are costly and often present added risks for patients; thus, patients and providers would benefit significantly from the development of an accurate noninvasive risk predictor of IPNs. Radiomic analysis of existing CTs presents a promising low cost alternative to invasive procedures. With radiomics, several features of a patient's IPN, such as the texture, density, and volume of the nodule are analyzed together to predict malignancy. We have begun running PyRadiomics, an open-source python package for the extraction of radiomic data from medical images, on IPNs from a cohort of patients treated at VUMC and the Tennessee Valley Veterans Affairs Healthcare System Nashville Campus (n = 170). The output of the algorithm's analysis will be used to develop a risk prediction model that will then be validated on external cohorts. Ultimately, we hope this model, when implemented alongside other noninvasive biomarkers, will result in clinically relevant improvements in IPN management and improved patient outcomes.

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30. Glucagon receptor antagonists to stimulate recovery of beta cells in diabetes.

Presenter: Alexandria Bustabad, University of South Florida

Program: Vanderbilt Diabetes Summer Research Program

Principle Investigator: Danielle Dean, Ph.D., Department of Medicine

Additional Project Authors: Tyler Rodgers, Katelyn Sellick, Madushika Wimalarathne, Department of Medicine

Abstract: Diabetes is a bihormonal disease with impaired insulin secretion from beta cells and glucagon secretion from alpha cells. Blocking glucagon receptor action in the liver using monoclonal antibodies (GCGR mAb) reduces glycemia in individuals with either type 1 or type 2 diabetes, but also results in

alpha cell hyperplasia in rodent models. In previous studies, GCGR mAb treatment of diabetic mice resulted in faster recovery of functional beta cell mass suggesting possible alpha to beta cell transdifferentiation or beta cell proliferation. We administered streptozotocin to induce diabetes in mice with fluorescently-labeled alpha cells (GcgCreERT2; tdTomatoFLOXSTOP) followed by six weeks treatment with GCGR mAb. Insulin-tomato double-positive cells were observed in both GCGR mAb and PBS diabetic islets with no large difference between treatment. Similarly, we saw little pS6 protein expression, a marker of mTOR signaling and cell growth, in insulin positive cells. Next, we used diphtheria toxin to induce beta cell loss in mice expressing the diphtheria receptor under the control of the rat insulin promoter followed by four weeks treatment with GCGR mAb. Again, insulin-glucagon double-positive cells were observed in both GCGR mAb and PBS diabetic islets with no large difference between treatment. Interestingly, glucose tolerance was improved in GCGR mAb treated DT-lesioned mice even 4 weeks after GCGR mAb withdrawal suggesting that blocking GCGR signaling may still have benefits that persist beyond acute treatment of diabetes although it is unclear if this is due to beta cell mass regeneration.

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31. Assessing the Gβγ-SNARE interaction at GABAergic synapses in the nucleus accumbens

Presenter: Sarah Cook, Yale University

Program: UCRIP

Principle Investigators: Brad Grueter, Ph.D., Department of Anesthesiology; Heidi Hamm, Ph.D., Department of Pharmacology

Project Mentor: José C. Zepeda, Department of Pharmacology

Abstract: The nucleus accumbens (NAc) transforms motivation into action via the complex integration of GABAergic, glutamatergic, and neuromodulatory inputs. Neuromodulators can bind to Gi/o-coupled G-protein-coupled receptors (GPCRs) to inhibit vesicular exocytosis onto NAc medium spiny neurons (MSNs). We recently showed that select GPCRs, including **μ** opioid receptors, can inhibit glutamate release via an interaction between Gβγ subunits and the t-SNARE protein SNAP25. However, it remains unknown whether the Gβγ-SNARE interaction is engaged by GABAergic synapses to exert effects on vesicular exocytosis. We hypothesized that Gβγ -SNARE is engaged by GPCRs on GABAergic synapses in the NAc and that compromising this interaction would disrupt basal release properties. We leveraged a transgenic mouse line which expresses a mutated SNAP25 protein (SNAP25D3) with compromised ability to bind to Gβγ combined with whole-cell voltage clamp electrophysiology to study whether GABAergic transmission engages the Gβγ-SNARE motif and whether GABAergic transmission properties are disrupted. We report that basal GABAergic transmission is not altered in SNAP25D3 mice and that **μ** opioid receptors do not dampen GABAergic transmission onto MSNs in the NAc. Dysfunction of neuromodulatory systems in the NAc have been strongly implicated in various neuropsychiatric disorders such as addiction, mood disorders and depression. Our results demonstrate that the same neuromodulatory system can vary in function between cell-types and synapse types.

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32. Loss of MCL-1 Disrupts Mitochondrial Cristae Morphology

Presenter: Madison Yarbrough, University of Central Arkansas

Program: Aspirnaut

Principle Investigator: Vivian Gama, Ph.D., Department of Cell and Developmental Biology

Project Mentor: Marina Hanna, Department of Cell and Developmental Biology

Abstract: Mitochondria are central regulators of calcium maintenance, metabolism, and energy production. Mitochondrial defects can lead to devastating neurodevelopmental diseases. Previous work in our laboratory found that inhibiting myeloid cell leukemia 1 (MCL-1) in human neural progenitor cells (hNPCs) leads to a loss of the essential transcription factor paired box protein 6 (PAX6) – suggesting a loss of cell identity that could hinder differentiation into lineage-specific cell types. MCL-1 is found at the outer and inner mitochondrial membranes (OMM and IMM, respectively). At the OMM, MCL-1 functions as an anti-apoptotic protein, inhibiting cell death effector proteins. However, its function at the IMM is not well understood. Previous research in our laboratory has found that MCL-1 at the IMM associates with the fusion protein, Optic Atrophy Type 1 (OPA-1), and the cristae shaping protein, Mitochondrial Contact Site and Cristae Organizing System (MICOS) – suggesting non-apoptotic functions of MCL-1. Using transmission electron microscopy (TEM), we show that inhibition of MCL-1 destabilizes cristae structure, which likely underlies loss of cell identity. This aligns with our findings that inhibition of MCL-1 in hNPCs decreased the expression of OPA-1 and MICOS complex proteins. Overall, MCL-1 appears to have a novel function at the IMM in hNPCs stabilizing the mitochondrial cristae structure. Determining the non-canonical role of MCL-1 in mitochondria will expand our knowledge of cristae structure and its effect on maintenance of multipotent stem cell identity.

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33. Exploring the regulatory mechanisms of the OAS-RNase L and PKR pathways in RNA sensing

Presenter: Channita Keuk, Sewanee: The University of the South

Program: V-SURE

Principle Investigator: John Karijolich, Ph.D., Department of Pathology, Microbiology, and Immunology

Project Mentor: Ruilin Zhang, Department of Pathology, Microbiology, and Immunology

Abstract: As dsRNAs are the hallmark of viral infections, RNA sensing becomes an important component of host innate immunity. Two dsRNA-sensing pathways are the oligoadenylate synthetase (OAS)-RNase L pathway and the protein kinase R (PKR) pathway. Given the potency of their activation, both pathways are not only highly regulated through cellular mechanisms but also heavily antagonized by viruses. To test whether regulation of the OAS/RNase L pathway occurs at the post translational level, RNase L wildtype and K684R mutant plasmids were generated, and the effect of acetylation of RNase L at residue 684 on RNase L activation was evaluated via bioanalyzer assays. One mechanism that the vaccinia virus (VacV) utilizes to induce host shut-off is the hypophosphorylation of serine/arginine-rich (SR) proteins which are essential to the host alternative RNA splicing machinery. To explore whether defects in splicing generates ligands for PKR activation, another scope of my project investigates the effect of SR inhibition on PKR activation. Completion of this project will enhance our mechanistic understanding of RNase L and PKR activation for RNA sensing.

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34. Evaluation of Cefiderocol comASP Against Reference Broth Microdilution

Presenter: Marina Saber Khalil, Middle Tennessee State University

Program: UCRIP

Principle Investigator: Romney Humphries, Ph.D., Department of Pathology, Microbiology, and Immunology

Project Mentor: Carmila Manuel, Department of Pathology, Microbiology, and Immunology

Abstract: Cefiderocol Susceptibility testing presents a major challenge for antibiotic susceptibility testing (AST) in clinical laboratories. Cefiderocol is a siderophore cephalosporin with activity against aerobic multidrug-resistant (MDR) gram-negative bacteria. Cefiderocol testing is complex, requiring iron-depleted Mueller–Hinton broth for growth. Testing options are limited and time-consuming to prepare in-house. Cefiderocol ComASP is a new diagnostic product that offers a more compact version of the Standard broth microdilution (BMD) testing. In this study, we evaluated 52 non-fastidious MDR gram-negative bacteria, including metalloβ-lactamase (MBL) producing bacteria, to assess their performance. Samples include Enterobacteriales (carbapenem-resistant), Pseudomonas aeruginosa, Acinetobacter baumannii complex, and Stenotrophomonas maltophilia. Known isolates were collected from three different sites JHH, VUMC, and CDC AR Bank to analyze. Categorical and essential agreements between Cefiderocol comASP and BMD reference standards were assessed according to the procedure outlined in M23. Despite the limited completed samples thus far in the study, data shows 92.5 and 32.6% categorical and essential agreement, respectively. Three minor and one major errors were present at 5.7 and 2.1%, both under the defined CLSI acceptable rates. Our preliminary results suggest that ComASP can be utilized to determine the susceptibility of isolates in clinical laboratories. However, further trials are needed in order to use ComASP in reporting an accurate minimum inhibitory concentration (MIC).

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35. Sodium Induced-RGMa Plays a Role In Salt-Sensitive Hypertension via TGFβ1 signaling of SMAD3

Presenter: Selam Desta, Howard University

Program: PAECER

Principle Investigator: Annet Kirabo, D.V.M., M.Sc., Ph.D., Department of Clinical Pharmacology

Additional Project Authors: Mohammad Saleem, Ashley Pitzer Mutchler, Lale Ertuglu, Department of Clinical Pharmacology

Abstract: Salt-sensitvity of blood pressure (SSBP), characterized by blood pressure fluctuations that mirror sodium (Na+) intake, is an independent risk factor for death due to cardiovascular disease. We previously established that Na+ transport through the epithelial sodium channel (ENaC) into antigen-presenting cells leads to production of immunogenic isolevuglandins. The precise mechanism by which this process unfolds remains unknown. Repulsive guidance molecule-a (RGMa) mediates SMAD3 activity during its interactions with TGFβR1 in immune activation, however, the role of this signaling pathway in SSBP is not known. We hypothesized that RGMa regulates SMAD3 through TGFβ1 signaling in SSBP. To test this, we isolated human monocytes (N=11) and treated them with high (190 mMol/L) or normal (150 mMol/L) Na+ in vitro and subsequently performed bulk RNA sequencing. Interestingly, RGMa and SMAD3 were significantly upregulated after high salt treatment compared to normal salt (2039±3640 vs.15.0±6.24, $p < 0.001$) and (2253±441.0 vs. 516±84.1, $p < 0.001$) respectively. To further investigate, we performed CITE-seq analysis after in vivo high Na+ treatment in humans utilizing a rigorous salt-loading/depletion protocol following an anti-hypertensive drug washout period. We did not find any correlation between increase in RGMa and systolic blood pressure ($r = 0.4683$, $p = 0.2418$). However, the correlation between increased SMAD3 expression and pulse pressure approached statistical significance ($r = 0.6541$, $p = 0.0785$). Our findings suggest that Na+ may modulate RGMa/SMAD3 signaling, and further studies may unveil a novel therapeutic target for SSBP treatment.

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36. Affect of Gene Programming in the offspring born of Diabetic Dams

Presenter: TyJanae Livers, Miles College

Program: PAECER

Principle Investigator: Rolanda Lister, M.D., Department of Obstetrics & Gynecology

Abstract: Maternal Diabetes has shown many signs of intrauterine anomalies that continue into the adult life of offspring. Infants of diabetic mothers are known to be more likely to have short-term and long -term cardiac anomalies including hypertension, cardiomyopathy, and cardiac hypertrophy. Abnormal gene programming may be the potential culprit in the genesis of these cardiac diseases that are seen in offspring.

The purpose of this study is to identify the correlation between differentially expressed genes and cardiac hypertrophy in adult animals that were born to diabetic dams. Dams were injected with Streptozotocin to induce diabetes before giving birth to offspring. Echocardiograms were used to measure the cardiac function of both control and diabetic dams. Our results suggest that increased Cacna1d is a candidate for mediating cardiac hypertrophy in adults born to diabetic mothers.
Our data support the hypothesis that alterations in levels of specific genes may be responsible for the development of cardiac hypertrophy.

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37. Heterogeneity in the expression of ADAMTS10 in transfected cells

Presenter: LilyJasmine Notice, Oakwood University

Program: Aspirnaut

Principle Investigator: Rachel Kuchtey, M.D., Ph.D., Vanderbilt Eye Institute

Project Mentors: John Kuchtey, Ph.D. and Samuel Insignares, Vanderbilt Eye Institute

Abstract: Glaucoma is a neurodegenerative disease and a leading cause of irreversible vision loss that affects tens of millions of people worldwide. It is characterized by the degradation and eventual apoptosis of retinal ganglion cells that form the inner-most layer of the retina, and whose axons form the optic nerve, which relays visual information to the brain. In humans, the most common form of glaucoma is primary open angle glaucoma (POAG), which is a complex disease associated with many genetic defects. In Beagle dogs, POAG has been established as an autosomal recessive disease, caused by a Gly661Arg mutation in ADAMTS10. Previous work has demonstrated that transient transfection of HEK293T cells did not induce adequate expression of ADAMTS10. To study the effect of the Gly661Arg mutation on the expression of ADAMTS10, we generated stably transfected HEK293T cells with a plasmid encoding mutant (8) or wildtype (N1) ADAMTS10. In contrast to transiently transfected cells, stably transfected HEK293 cells that were cultured for an extended period demonstrated heterogeneity in their expression of ADAMTS10. Use of epitope (FLAG)-tagged ADAMTS10, which we detected using an anti-FLAG antibody and fluorescent immunohistochemistry, demonstrated that very few cells expressed FLAG-tagged-ADAMTS10 and that those cells had a wide variance in fluorescence intensity (420% and 470% of the mean, respectively). The average fluorescence intensity was not significantly different between wildtype and mutant transfected cells.

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38. Novel Function of Endocannabinoid System and Prostaglandin-Glycerols on Cytokine Release in LPS-Activated Macrophages

Presenter: Kaziah Vaughn, University of Vermont

Program: Aspirnaut

Principle Investigator: Lawrence Marnett, Ph.D., Departments of Biochemistry, Chemistry, and Pharmacology, Vanderbilt Institute of Chemical Biology, and Vanderbilt-Ingram Cancer Center

Project Mentor: Philip Kingsley, M.A., Departments of Biochemistry, Chemistry, and Pharmacology, Vanderbilt Institute of Chemical Biology, and Vanderbilt-Ingram Cancer Center

Additional Project Authors: Robin Richie-Jannetta, Ansari Aleem, Carol Rouzer, Departments of Biochemistry, Chemistry, and Pharmacology, Vanderbilt Institute of Chemical Biology, and Vanderbilt-Ingram Cancer Center

Abstract: Activated macrophages can release cytokines which are signaling molecules that further activate and regulate the immune response. When activated by bacterial peptide lipopolysaccharide (LPS), RAW246.7 macrophage-like cells express the enzyme cyclooxygenase-2 (COX-2). COX-2 metabolizes arachidonic acid (AA) and the endocannabinoid 2-arachidonoylglycerol (2-AG), resulting in the early formation of prostaglandins (PGs) and a latent release of prostaglandin-glycerols (PG-Gs), respectively. However, the role of PG-Gs in the immune response is unknown. Here we demonstrate the impact of PG-Gs on cytokine release in LPS-activated macrophages. Using a capture ELISA, we demonstrate that when PG-G synthesis is inhibited, it induces an increase in the concentration of monocyte chemoattractant protein-1 (MCP-1). MCP-1 is a cytokine that recruits other immune cells to a site of infection. The increase in MCP-1 concentration in the context of PG-G inhibition is indicative of PG-Gs' role in regulating the immune response. This research suggests a novel role of 2-AG and resultant PG-G synthesis on the immune response. This connection between the endocannabinoid system and the immune response demonstrates a potential for cannabinoid-targeted and cannabinoid-derived therapeutics.

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39. Chaperoning Protein-Folding Stability of Src Family Kinases Using Hsp90

Presenter: Clarise Guadalupe Rivera, University of California, Berkeley

Program: Independent Research Intern

Principle Investigator: John Kuriyan, Ph.D., Department of Biochemistry, Vanderbilt University

Project Mentors: Joseph Paul, Department of Molecular and Cell Biology, Department of Chemistry, University of California, Berkeley. Serena Muratcioğlu Ph.D., Department of Biochemistry, Vanderbilt University

Abstract: Src family kinases (SFKs) are an eight-membered group of non-receptor protein tyrosine kinases which play an important role in cell proliferation, differentiation, and metabolism. In order to function properly, SFKs need to fold in a manner that prevents the formation of protein aggregates. One particular chaperone protein, known as Heat Shock Protein 90 (Hsp90), mediates aggregates by adhering to the kinase domain (KD) of SFK and chaperones the protein until a stable folding-response is generated.

The mechanism by which Hsp90 stabilizes SFKs remains unclear. After using crystal structures of two SFK members, Lck and Src, we superimposed and observed a nearly identical domain structure with

contrasting Hsp90 interaction score stability. To study this phenomenon, we used Lck and Src KDs to generate viruses to transduce SFK plasmids. Using Jurkat T-cells, we treated both Lck and Src KDs with Hsp90-inhibtor (Hsp90i) to evaluate the stability of T-cell signaling under the absence of chaperone-mediated protein-folding. Cells were then sorted using a fluorescently activated cell sorter expressing two fluorescent sensors: mTagBFP2 serves a control for fluorescent oscillations latched onto the kinase domain, and mNG serves as a degron for Hsp90.

As a result, Lck KD demonstrated more sensitivity to Hsp90i than Src KD, suggesting the kinase domain of Lck is less stable and relies on Hsp90 for stability. These results will help to understand the evolutionary differences between kinases and how they're regulated under Hsp90.

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40. Validation of Camelid Nano-Bodies for imaging Alzheimer's Disease

Presenter: Miles Carter, New York University

Program: BP-ENDURE

Principle Investigator: Wellington Pham, Ph.D., Department of Imaging Science

Additional Project Authors: Charlotte A. Landman, Justin R. Haynes, William J. Behof, Kenny Tao, Brian E. Wadzinski Benjamin W. Spiller

Abstract: Alzheimer's is a brain disorder that impairs memory and thinking ability by disrupting the communication of neurons. Alzheimer's diseased brains have an abnormally high amount of a protein known as beta-amyloid, which clump together to form plaques. As these plaques build up in the extracellular space, they can disrupt communication between neurons. The Pham Lab has been utilizing specific antibodies known as 'nanobodies' to help visualize these plaques. Nanobodies are around 10x smaller than regular nanobodies and can pass through the blood brain barrier. This project had two goals, to validate specificity of an anti-soluble Beta-Amyloid oligomer nanobody and Imaging soluble Beta-Amyloid oligomer flow dynamics in the retina. The project first involved performing a western blot comparing our regular E3 nanobody to the labeled E3 nanobody probe, to ensure we had the correct product. Once we confirmed we had the product we needed. Before we imaged we performed an intravenous injection of 150uL of our labeled E3 probe at a concentration of 20mg/kg via lateral tail vein of a 5XFAD (Alzheimer's diseased) mouse. Immediately after the injection we performed retinal imaging with a Scanning Laser Ophthalmoscopy device which allowed us to observe our probe through the lens of the eye in the vasculature. We were successful in our retinal imaging and could observe our nanobody in the vasculature and capillaries of the retina. We hope to apply this protocol to observe differences in the beta amyloid oligomer flow dynamics between wildtype and 5XFAD mice and produce a more effective method to detect these oligomers/plaques in the retina.

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41. Identifying Genetic Mutations Involved in *CTLA-4* Haploinsufficiency

Presenter: Elijah Eshaun Burks, Tulane University

Program: MSTP Summer Research Program

Principle Investigator: Janet Markle, Ph.D., Department of Pathology, Microbiology, and Immunology

Project Mentor: James Maiarana, M.D., Department of Pediatrics

Abstract: Inborn errors of immunity (IEI) are monogenic diseases that can cause lymphoproliferation, autoinflammation, autoimmunity, allergy, and malignancy. A total of 485 IEIs affect every 1 in 1000 to 5 in 1000 births, and IEIs are expanding due to improvements in genetic sequencing.

One IEI is *CTLA4* haploinsufficiency, a rare autosomal dominant disorder characterized by lymphoproliferation, hypogammaglobulinemia, and recurrent infections. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is a protein found on activated T cells and constitutively on regulatory T cells. CTLA-4 is critical in T cell regulation, competing with CD28 for binding to costimulatory CD80/86. CTLA-4 has a higher affinity for CD80/86 and inhibits the CD28-CD80/86 signaling by transendocytosing CD80/86. This removes CD80/CD86 from the cell surface, limiting ligand availability.

One known pathogenic variant, R51X, halts transendocytosis and presents clinical features of *CTLA4* haploinsufficiency. Two Variants of unknown significance in *CTLA4* were determined from patients, and we sought to test if those variants (Y139S and G142R) were pathogenic.

Chinese Hamster Ovarian (CHO) cells were transfected with the WT and R51X controls and genetic variants Y139S and G142R and allowed to undergo transendocytosis with CHO-CD80^{GFP} cells. Flow cytometry revealed that Y139S and G142R had decreased percentages of GFP expression compared to WT.

These findings demonstrate that Y139S and G142R functionally decrease transendocytosis and may be pathogenic variants of *CTLA4*. This research can streamline the diagnosis and care of immune diseases and provide more precise immunotherapy treatment for patients with *CTLA4* haploinsufficiency.

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42. Cigarette Smoke Induces Pulmonary Vascular Remodeling Through Formation of Isolevuglandins and Sirtuin-3 Inactivation

Presenter: Shaelyn Walker, Duquesne University

Program: Aspirnaut

Principle Investigator: Vasiliy Polosukhin, M.D., Ph.D., Department of Medicine, Division of Allergy, Pulmonary, and Critical Care Medicine

Project Mentor: Sergey Gutor, Ph.D., Department of Medicine, Division of Allergy, Pulmonary, and Critical Care Medicine

Additional Project Authors: Isabella Gaona (1), Anna Dikalova (1), Timothy Blackwell (2), Department of Medicine, Division of (1) Allergy, Pulmonary, and Critical Care Medicine, (2) Department of Medicine, Division of Clinical Pharmacology

Abstract: Exposure to cigarette smoke (CS) initiates pulmonary vascular (PV) remodeling prior to lung function decline, suggesting that PV remodeling could be a direct response to CS. The most prominent feature of PV remodeling in smokers is intima and media thickening in arteries with a diameter of less than 500 µm; however, the mechanisms by which CS promotes PV remodeling are unclear. Here we identify a role for mitochondrial isolevuglandins (isoLGs) and hyperacetylation of the mitochondrial antioxidant, superoxide dismutase-2 (SOD2) in CS-induced oxidative stress and PV remodeling. Blinded histomorphometric analysis of lungs from wild-type mice exposed to CS or ambient air twice a day for 2 months (8 mice per study group, C57Bl/6J) did not identify a difference in mean linear intercept between exposed and unexposed mice. However, pulmonary arteries of mice exposed to CS demonstrated abnormal thickening of the tunica media (7.4±0.6µm and 6.3±0.4µm for CS-exposed and unexposed mice, respectively, $p<0.05$) and the tunica intima (0.7±0.2µm and 0.4±0.1µm for CS-exposed

and unexposed mice, respectively, $p<0.05$) as well as a higher percentage of muscularized vessels with diameter <25µm. Western blots of whole lung lysates indicated that CS exposure induced the formation of toxic lipid peroxidation products, particularly isoLGs, and caused hyperacetylation of SOD2. Treatment of CS-exposed mice with the isoLG scavenger 2-HOBA abrogated PV remodeling. Moreover, sirtuin-3 overexpressing mice, which demonstrate attenuation of SOD2 acetylation, were also protected from CS-induced PV remodeling. These findings suggest that scavenging of mitochondrial isoLGs has therapeutic potential in treatment of PV dysfunction and remodeling.

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43. Targeting Notch1 and 5-HT2B Pathways: Novel Therapeutic Approaches for Calcific Aortic Valve Disease and Mitral Valve Regurgitation.

Presenter: Gordina Princess Hodibert, University of Virginia

Program: PAECER

Principle Investigator: David Merryman, Ph.D., Biomedical Engineering

Project Mentors: Oluwalade Ogungbesan, Angela Totoro, PhD for Biomedical Engineering

Abstract: In the United States, approximately 2.5% of the population is affected by valvular disease. Calcific aortic valve disease (CAVD) and mitral valve regurgitation (MR) are the prevalent conditions that contribute to this statistic. Our research aims to explore how specifically relevant pathways affect the incidence of developing CAVD and MR. Investigating CAVD, we used siRNA transfection and western blotting to examine the expression levels of cadherin-11 in aortic valve interstitial cells. The elevated risk of developing MR was assessed using echocardiography and qPCR, which allowed us to study the potential association with increased serotonin 2B (5-HT2B). Understanding the interaction between Cadherin-11 and Notch1 signaling in CAVD and 5-HT2B in MR provides crucial insights into the diseases' pathology. These insights may lead to new therapeutic strategies for managing these cardiovascular conditions. Results are ongoing.

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44. Asn211Gln Glycosylation Site of Discoidin Domain Receptor 1 Resulting in Ligand Independent Signaling

Presenter: Oscar Hanson, Berea College

Program: Aspirnaut

Principle Investigator: Ambra Pozzi, Ph.D., Department of Medicine, Division of Nephrology and Hypertension, Department of Veterans Affairs

Project Mentor: Gema Bolas, Ph.D., Department of Medicine, Division of Nephrology and Hypertension

Abstract: Discoidin Domain Receptor 1 (DDR1) is a receptor tyrosine kinase (RTK) that is activated by collagens in the extracellular matrix. Upon collagen activation, DDR1 undergoes tyrosine auto-phosphorylation on its kinase domain (KD), initiating various downstream signaling pathways vital for physiological and pathological functions such as cell migration, extracellular matrix remodeling and production, and inflammatory cytokine secretion. Hence, the regulation of DDR1 activation is critical to prevent DDR1-mediated pathological effects. The N-glycosylation amino acid Asn211 in the extracellular Discoidin-like (DS-like) domain of DDR1 has been found to play a role in maintaining the inactive state of the KD. Particularly, the mutation Asn211Gln has been shown to constitutively activate the tyrosine kinase. Though, whether ligand independent DDR1 activation contributes to downstream signaling is yet

to be determined. Here, we show that the Asn211Gln mutant DDR1 undergoes autophosphorylation independent of collagen stimulation as compared to the ligand dependent activation of wild-type DDR1. Hence, impairing the N-glycosylation site of the DS-like domain results in the constitutive activation of DDR1 and its downstream signaling.

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45. Effects of antibacterials on DNA cleavage mediated by *Pseudomonas aeruginosa* topoisomerase IV

Presenter: Kayla Hardrick, Miles College

Program: Aspirnaut

Principle Investigator: Neil Osheroff, Ph.D., Department of Biochemistry, Department of Medicine

Project Mentor: Jillian Armenia, Department of Biochemistry

Abstract: *Pseudomonas aeruginosa* is a gram-negative, aerobic bacterium that causes a variety of infections and affects ~32,000 people each year. It an an opportunistic pathogen that primarily affects people who have pre-existing disorders that make them prone to infections, such as patients with HIV/AIDS, cystic fibrosis, and individuals in intensive care units who require ventilators. *P. aeruginosa* can cause post-operative wound infection in addition to causing lung disease in the aforementioned populations. Antibacterial resistance in *P. aeruginosa* has become a significant threat to healthcare, severely limiting the ability to treat infected patients. Potential targets for antibacterial drugs that overcome resistance are the bacterial type II topoisomerases, gyrase and topoisomerase IV. These enzymes modulate the topological state of DNA in the cell by generating transient double-stranded breaks in the double helix. In this study, we investigated the ability of different antibacterials that target type II topoisomerases to enhance DNA cleavage by P. Aeruginosa topoisomerase IV. Moxifloxacin is a fluoroquinolone, zoliflodacin is a spiropyrimidinetrione, and gepotidacin is a triazaacenaphythlene-based novel bacterial topoisomerase inhibitor (NBTI). Moxifloxacin primarily increased topoisomerase IV-mediated double-stranded DNA breaks. Similar to other NBTIs, gepotidacin increased single-stranded breaks in plasmid substrates following in vitro exposure to topoisomerase IV in the presence of the antibacterials. Investing type II topoisomerases as potential drug targets for *P. aeruginosa* paves the way for the development of new therapeutics to treat drug resistant bacterial infections.

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46. Understanding the Effects of Mitochondrial Trans-2-Enoyl-CoA Reductase (Mecr) on CD4+ T Cells Cytokine Expression

Presenter: Richmond Okparaugo, Philander Smith College

Program: Aspirnaut

Principle Investigator: Jeffrey Rathmell, Ph.D., Department of Pathology, Microbiology, and Immunology

Project Mentor: KayLee Steiner, Program in Cancer Biology

Abstract: The enzyme, Mecr, has emerged as a subject of growing interest in immunology research because of its role in mitochondrial fatty acid synthesis (mtFAS). Mecr catalyzes the reduction of 2-enoyl CoA intermediates to acyl-CoA derivatives, contributing to the oxidative phosphorylation (OXPHOS). This process is important for the generation of various lipids necessary for cell function and metabolism and may also contribute to electron transport chain stability. Under normal conditions, cells derive ATP from metabolic breakdown of glucose via glycolysis, OXPHOS, and electron transport chain. Previous work

shows that cells cultured in galactose rely mostly on OXPHOS for ATP. However, to our knowledge, there has been no study to compare the effects of Mecr on CD4+ T-cells in galactose and glucose media. Here we show that Mecr does not affect transcription factor or cytokine expression by CD4+ T cells cultured in the presence of galactose or glucose. We used CRISPR/Cas9 to knock out the Mecr gene from CD4+ T-cells in Cas-9 transgenic mice and cultured Mecr-knockout and non-targeting control (NTC) CD4+ T-cells in media with glucose or galactose. Following stimulation of the cells to produce cytokines, no significant differences were observed in the expression of IL-2, IFNg, TNFa+, between NTC and Mecr knockout cells under either culture condition. This suggests that Mecr is crucial in other cell functions like mtFAS and OXPHOS, however, it plays no role in cytokine expression.

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47. Interaction between NTF2L Domain of NXF1-NXT1 Export Receptor and CTE RNA

Presenter: Hira Karim, Berea College

Program: Aspirnaut

Principle Investigator: Yi Ren, Ph.D., Department of Biochemistry

Project Mentor: Menghan Mei, Department of Biochemistry

Additional Project Authors: Bradley Clark, Ph.D., Department of Biochemistry

Abstract: Human nuclear export of messenger RNAs (mRNAs) is facilitated by the principal mRNA export receptor, NXF1-NXT1. Following their synthesis in the nucleus, fully processed mRNAs are loaded with NXF1-NXT1 by mRNA export machinery and translocated through the nuclear pore complex into the cytoplasm for gene expression. Retroviruses exploit this nuclear export machinery to export their unprocessed viral mRNA to the host cytoplasm. Maison-Pfizer Monkey virus, and other Simian type D retroviruses, export their unprocessed mRNA by binding a small part of their mRNAs called the constitutive transport element (CTE) to host NXF1-NXT1 receptors. NXF1 has 3 main domains, RRM, LRR and NTF2L, which are all capable of binding to RNA. The RRM and LRR domains have been shown in crystal structure, to bind to CTE RNA, but whether the NTF2L domain binds to CTE RNA is still unknown. Here we investigate the potential molecular interaction between the NTF2L domain and CTE RNA by introducing para-Benzoylphenylalanine (pBpa), a photoactive unnatural amino acid, to different locations within the NTF2L domain of NXF1. We then induce photo-crosslinking between NXF1-NXT1 and CTE RNA with UV radiation, and the crosslinked products are analyzed by SDS-PAGE and fluorescence imaging.

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48. Investigating Two SCN5A Variants with Discordant In Vitro Results and Patient Phenotypes

Presenter: Renaya Imani Kelly, North Carolina Agricultural and Technical State University

Program: PAECER

Principle Investigator: Dan Roden, M.D., Ph.D., Department of Medicine

Project Mentor: Andrew Glazer Ph.D., Department of Medicine

Additional Project Authors: Yuko Wada, Jeremy E. Smith, Julie A. Laudeman, M. Lorena Harvey, Joseph F. Solus, Department of Medicine

Abstract: SCN5A is a gene that corresponds to a cardiac sodium channel. Loss of function variants in SCN5A can cause inherited heart arrhythmias. Previous studies of SCN5A by our group found two variants, S216L and R1644C, with differing in vitro results and patient phenotypes. We created multiple versions of S216L and R1644C on different SCN5A wildtype backgrounds using restriction digestion, ligation, and transformation. We integrated these plasmids into Human Embryonic Kidney (HEK293) cells. We cloned guide RNA plasmids to perform CRISPR editing of S216L and R1644C and inserted them into induced pluripotent stem cells (iPSC) followed by automated patch clamping using the SyncroPatch 384 PE robotic patch clamp system. Future work by the lab will differentiate these iPSCs into cardiomyocytes and measure sodium currents by patch clamping. Distinct functions of S216L or R1644C in different wildtype backgrounds by automated patch clamping will lead us to conclude that this difference underlies the discordant in vitro and patient phenotype data. If we identify a difference in variant function in iPSC-derived cardiomyocytes, we will conclude that these variants have a different function in iPSC-CMs compared to HEK293 cells.

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49. Analyzing Longitudinal Measures of Anxiety and Depression to Inform Clinical Outcomes

Presenter: Leila Ghaffari, University of Maryland, Baltimore County

Program: MSTP Summer Research Program

Principle Investigator: Doug Ruderfer, Ph.D., Department of Medicine

Abstract: Our daily stresses and activities seem to be affecting not just our mental health, but our behavior may have significant consequences on our overall health. A correlation is shown between behavior and clinical outcomes and diagnoses, such as heart attacks and obesity diagnoses. The goal of our project is to understand the relationship between CAT-MH data, which are surveys of depression and anxiety, and Electronic Health Records (EHR) which allow us to characterize patient population in the Vanderbilt University Medical System and diagnostic codes. This project focusses on anxiety and depression specifically, and major categories of clinical diagnoses, like diabetes and heart disease. CAT-MH data includes 257 unique individuals, and there are 222 unique individuals in the EHR. Using Python code, we look at our data in two ways. The first looks at individuals with diagnoses in EHR have participated in at least one CAT-MH survey. We hope to understand the relationship between the survey and the diagnoses, elucidating how many people are involved in both clinical and mental health instances. The second approach looks at individuals with their first diagnostic code six months from their initial CAT-MH survey. We will use a cox regression to interpret the significance of any relationship between the severity reported in the CAT-MH survey and the time elapsed to the patients' first EHR diagnoses six months later. With this data we hope to quantify and characterize how anxiety and depression has a correlation to occurrence of clinical outcomes and the placement of a diagnoses.

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50. Fragile human red blood cells collected during sepsis exhibit faster shape recovery than red blood cells from healthy donors.

Presenter: Eric Joseph Schall, Lipscomb University

Program: UCRIP

Principle Investigator: Ciara Shaver, M.D., Ph.D., Department of Allergy, Pulmonary, and Critical Care Medicine

Project Mentor: Nancy Wickersham, Department of Allergy, Pulmonary, and Critical Care Medicine

Additional Project Authors: Julie Bastarache, Department of Allergy, Pulmonary, and Critical Care Medicine

Abstract: Background: Patients with sepsis have increased red blood cell (RBC) fragility that causes release of cell-free hemoglobin and organ injury. Why RBCs are more fragile in sepsis is unknown. We hypothesize that septic blood will have reduced RBC deformability and increased time for deformed RBC shape recovery compared to healthy blood.

Methods: We measured RBC deformability and shape recovery (MIZAR, ALCOR Scientific, Smithfield, RI) of RBCs under shear stress conditions. We collected whole blood from subjects with sepsis within 24 hours of ICU admission (n=17) and healthy control subjects (n=7). To test if changes in RBC fragility was due to bacterial exposure, healthy whole blood was incubated with LPS (0-200nM), and RBC deformability and shape recovery were measured. Groups were compared using Mann-Whitney U test.

Results: There was no difference in RBC deformability between septic patients and healthy controls (septic, mean deformability 59.7 +/- 16.1; control 50.6 +/- 9.8, p=0.105). In contrast, the shape recovery of RBC was significantly faster in septic patients (septic, mean time for shape recovery 186 +/- 38 msec; control 263 +/- 50, p=0.003). LPS exposure for up to 24h in vitro did not change RBC deformability or shape recovery.

Conclusions: These data suggest that sepsis changes RBC shape recovery without changing RBC deformability. LPS exposure is insufficient to explain this observation. One possible mechanism to link shape recovery and hemolysis in sepsis would be membrane degradation from inflammatory mediators. Further research is needed to better understand mechanisms of shape recovery and RBC lysis during sepsis.

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51. Optimization of a High-throughput Screening Assay for Drug Discovery against Infection-induced Inflammation Leading to Preterm Birth

Presenter: Madeline Jones, Lawrence University

Program: UCRIP

Principle Investigator: Shajila Siricilla, Ph.D., Department of Neonatology

Additional Project Authors: Alexus J. Brown

Abstract: Preterm birth (PTB), birth before 37 weeks gestation, affects 10% of newborns annually in the US and is a leading cause of death for children under 5 years old. While not all the causes of PTB are known, literature has established that an inflammatory signaling cascade (mediated by IL1b, IL6, TNFa and others) initiated by bacterial infections in fetal membranes (FMs) is known cause of early labor. The goal of this research was to develop a large-scale assay for high-throughput screening (HTS) of small-molecules, that inhibit the release of the major proinflammatory cytokines in FM explants stimulated with pathogen-associated molecular patterns (PAMPs) of bacteria associated with PTB.

FMs were collected from placentas of consenting patients (>39 weeks) scheduled for c-section. FM biopsies(4mm) were incubated overnight in 96-well plates with media (penicillin-streptomycin, 37C, 5%CO2). Biopsies were then exposed to PAMPs (LPS, PG, PAM3, and FSL1: 4.6ng/mL to 10ug/ml) and control molecule TPCA1(IKK inhibitor). Time-course and concentration-response were determined for each PAMP (0,4,6,8 and 24hrs). Released cytokines (IL1b, IL6, TNFa) were quantified using Luminex

assay. Total protein concentration in each sample was quantified using a bicinchoninic acid(BCA) assay and normalized to cytokines levels.

PAMPs dose-dependently stimulated IL6 and TNFa-release after 6,8,24hrs, and IL1b at 24hrs. TPCA1 inhibited all cytokines at all PAMPs concentrations examined. Collectively, these studies demonstrate a sensitive assay to measure changes in proinflammatory cytokine release from human FMs in 96-well format. Future studies will be directed towards large-scale HTS against small-molecule libraries to identify novel inhibitors of inflammation-induced PTB.

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52. Novel Pathology Report to Potentially Improve Communication between Surgeons and Pathologists

Presenter: Yuna Chung, University of Richmond

Program: UCRIP

Principle Investigator: Michael Topf, M.D., Department of Otolaryngology

Additional Project Authors: Liyu Huang, Spencer Yueh, Kyle Mannion, Michael Topf, Department of Otolaryngology

Abstract: The pathology report is an important tool used to communicate surgical results between surgeons and pathologists. To create this report, surgical pathologists perform gross and microscopic analysis of surgical specimens and organize their findings in a written document. The information provided in the report influences the patient's diagnosis and treatment. Given the complex three-dimensional (3D) nature of many surgical specimens, physicians have reported difficulty interpreting the written pathology report without a visual aid. To address this, our team has developed a novel pathology report that includes photorealistic structured-light 3D maps of surgical specimens. This study explores the feasibility of utilizing this novel pathology report in oncologic surgery. Surgical specimens are 3D scanned per a previously published protocol. The virtual 3D model is virtually marked, or mapped, using computer-aided design (CAD) software alongside the pathology team to accurately indicate sectioning and inking of the specimen. The 3D maps are then incorporated into the pathology report for an easy to understand, visual description of the specimen. Sample pathology reports were created for a diverse array of cases which include a scalp resection and craniectomy for advanced skin cancer, partial mastectomy for breast cancer, chest wall resection sarcoma, and oral cavity composite resection for oral cavity cancer. This report has been shown to be applicable in a wide variety of cases, thereby suggesting the feasibility of its widespread incorporation.

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53. Protein Kinase D1 Mediates NCC Phosphorylation

Presenter: Kate Ifeoma Ejimogu, University of Maryland Baltimore County

Program: PAECER

Principle Investigator: Andrew Terker, M.D., Ph.D., Department of Nephrology

Abstract: Over 1 billion people in the world suffer from hypertension. Located in the distal convoluted tubule (DCT), the sodium chloride cotransporter (NCC) maintains blood pressure by reabsorbing sodium into the bloodstream. Phosphorylation is a key regulator of NCC, although the exact mechanism by which this occurs is unclear. In the DCT, Protein Kinase D1 (PKD1) is a kinase in which mRNA is highly expressed. We tested the hypothesis that PKD1 phosphorylates NCC. Using immunofluorescence co-

staining with NCC, we first verified the presence of PKD1 protein in the DCT. We next isolated mouse kidney tubules in varying culture mediums and inhibited PKD1 with the kinase inhibitor, CRT0066101, to examine the impact of PKD1 function on NCC. Compared to tubules cultured in 6 mM potassium, decreased potassium environments (0 mM potassium), a known activator of NCC phosphorylation, caused a rise in NCC phosphorylation (T53) shown through Western blot analysis. Inhibition of PKD1 in the tubules prevented the increase in NCC phosphorylation. Evidence from our work suggests PKD1 affects NCC phosphorylation and activation in the kidneys.

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54. The advent of LLMs in Medical Education: A New Age

Presenter: Harrison C. Lucas, Brandeis University

Program: UCRIP

Principle Investigator: Jeffrey Upperman , M.D., Department of Pediatric Surgery

Additional Project Authors: Dr. Jamie Robinson, Department of Pediatric Surgery

Abstract: Over the past year, the use of large language models (LLMs) has created a lot of interest and excitement. These models have the potential to revolutionize various fields, including medical education and support for aspiring physicians. Medical students must go through a rigorous and demanding education process to become competent healthcare professionals. However, the emergence of LLMs has offered a promising solution to some of the challenges faced by medical students such as exponential growth in new information, limited time, and busy bottom-line pressure on clinical educators. Nonetheless, integrating LLMs into medical education has raised some critical concerns and challenges for educators, professionals, and students. This scholarly perspective aims to explore the applications, benefits, headwinds, and future directions of LLMs in medical education, with a focus on their impact on medical students' learning experiences. By analyzing existing literature and research, we provide an overview of the current state of knowledge, identify the potential benefits and limitations of large language models in medical education, and highlight areas that require further investigation and development.

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55. Mechanisms of α-Parvin in DNA repair during kidney ureteric bud branching morphogenesis

Presenter: Hugo Arce-Santamaria, Berea College

Program: Aspirnaut

Principle Investigator: Roy Zent, Ph.D., Department of Medicine, Division of Nephrology and Hypertension

Project Mentor: Xinyu Dong, Department of Medicine, Division of Nephrology and Hypertension

Abstract: Integrins are extracellular matrix (ECM) receptors that regulate various cellular processes such as cell adhesion, proliferation, and apoptosis. These receptors transmit signals by recruiting scaffold proteins, which also link integrins to the actin cytoskeleton. One scaffold protein, α-Parvin, binds to F-actin but also forms a complex with Integrin-linked kinase (ILK) and Pinch (IPP complex). Previous studies have shown in certain cell lines that the loss of any component of the α-Parvin–ILK–Pinch complex leads to the degradation of the others. Our lab has previously shown that deletion of α-Parvin in the ureteric bud during kidney development results in defective branching morphogenesis and a decrease in cell

proliferation. We also observed a significant increase in protein levels of γ-H2AX, an indicator of DNA damage, in the absence of α-Parvin; however, the role of α-Parvin in DNA damage and repair is unclear. Here we identify a direct role of α-Parvin in DNA damage and repair in the nucleus that is independent of the IPP complex. In fractionated collecting duct (CD) cells, we first show that α-Parvin is found in both the nuclear soluble and the chromatin-enriched fraction. Surprisingly, we did not find a loss of ILK or Pinch protein in total, cytosolic, or nuclear fractions in α-Parvin-null CD cells, suggesting that the absence of α-Parvin does not affect the protein expression or nuclear-localization of ILK and PINCH. Further investigations on α-Parvin's critical role in DNA repair may enhance regenerative therapies for kidney tissue repair.

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56. Liraglutide Pretreatment Attenuates Sepsis-Induced Acute Kidney Injury

Presenter: Chrystal Omonzele Aluya, Harvard University

Program: UCRIP

Principle Investigator: Lorraine Ware, M.D., Department of Medicine, Allergy, Pulmonary & Critical Care Division

Project Mentor: Brandon Baer, Ph.D., Department of Medicine, Allergy, Pulmonary & Critical Care Division

Abstract:

Background: Acute kidney injury (AKI) is a common complication of sepsis and is associated with high mortality rates. Preliminary studies show that liraglutide, a diabetes medication and glucagon-like peptide-1 (GLP-1) receptor agonist, protects mice from death and acute lung injury in a model of murine sepsis. We hypothesized that liraglutide would also attenuate murine sepsis-induced AKI.

Methods: Sepsis was induced by intraperitoneal injection of cecal slurry (CS; 2.4mg/g) or 5% dextrose (control) followed by exposure to hyperoxia (HO; FiO2=0.95) or room air (control, FiO2=0.21). Mice were pretreated twice daily with subcutaneous injections of liraglutide (0.1mg/kg) or saline for 3-days prior to initiation of CS+HO. At 24-hours post-CS+HO, animals were sacrificed, kidney tissue was collected and homogenized. Kidney injury was assessed through neutrophil gelatinase-associated lipocalin (NGAL) and Kidney Injury Molecule-1 (KIM-1) expression via qPCR (mRNA) as well as Western blot (protein). Statistical analysis was performed using one-way analysis of variance with post hoc Tukey test.

Results: Among CS+HO mice, liraglutide reduced NGAL mRNA by 1.9-fold (CS+HO, 3446.9 + 482.4 2-ΔΔCT, p=0.002; N=5-20) and protein expression by 2.5-fold (CS+HO, 250342.9 + 52246.2 RE normalized to total protein; p=0.0357; N=4). Liraglutide did not affect KIM-1 mRNA expression (p=0.8397; N=5-20).

Discussion: These findings suggest that GLP-1 receptor activation may be a novel treatment strategy for prevention of sepsis-induced AKI. However, additional studies are needed to better understand its mechanism of action.

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57. Investigating the Role of Trained Immunity in *Tet2-/-* and *Tet2-/- RIPK1D/+* Bone Marrow Cells

Presenter: Kaiwen Zheng, Sewanee: The University of the South

Program: V-SURE

Principle Investigator: Sandra Zinkel, M.D., Ph.D., Department of Hematology Oncology

Project Mentor: Alyssa Jarabek, Molecular Pathology & Immunology Graduate Program

Abstract: Myelodysplastic syndromes (MDS) are a group of hematopoietic diseases characterized by clonal hematopoiesis, cytopenia, and dysplastic morphology. Inactivating mutations in the *Tet2* gene are present in 25% of patients with MDS, but also in healthy individuals over the age of 70, indicating that *Tet2* loss is not sufficient to cause disease. Innate immune activation can promote disease progression in the setting of *Tet2* loss. Our preliminary data demonstrate that increased innate immune inflammation in *Tet2*-deficient macrophages could be improved by inhibiting RIPK1, the major kinase driving inflammatory necroptotic cell death. We hypothesize that *Tet2* loss impacts the long-term response of the innate immune system to stimulation by pathogens, known as trained immunity. Trained immunity arms the innate immune system to elicit a more robust response upon additional exposure of pathogens. Here we investigate the response of wild-type, $Tet2^{-/-}$, or $Tet2^{-/-}$ $RIPK1^{D/+}$ bone marrow derived macrophages (BMDMs) to inflammatory stimuli. We quantified cytokine production via qRT-PCR in β-glucan (a fungal cell wall component) or monophosphoryl lipid A (MPLA, a less toxic derivative of lipopolysaccharide, or LPS) pretreated macrophages, which induces a trained immune response. We also stimulated total bone marrow cells with phorbol-12-myristate-13-acetate (PMA) or LPS and quantified cytosolic and mitochondrial ROS production by flow cytometry. We find decreased TNF cytokine expression in β-glucan and MPLA-trained cells upon LPS stimulation and changes in cytosolic and mitochondrial ROS production across various treatments and genotypes. Overall, our results suggests that *Tet2* and RIPK1 may play important roles in inflammatory responses via ROS production.

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58. MICOS Complex and Mitochondria Morphology Changes Across Aging in Cardiac Muscle

Presenter: Christopher Thomas Altamura, Stony Brook University

Program: PAECER

Principle Investigator: Antentor Hinton, Jr., Department of Molecular Physiology and Biophysics

Project Mentor: Zer Vue, Department of Molecular Physiology and Biophysics

Additional Project Authors: Andrea G. Marshall, Heather K. Beasley, Larry Vang, Kit Neikirk, Department of Molecular Physiology and Biophysics; Edgar Garza Lopez, Department of Internal Medicine, University of Iowa

Abstract: Background: Cardiac disease remains a significant cause of death among humans and therapies to treat the disease are lacking. Importantly, heart failure has also been linked to factors including endoplasmic reticulum stress, mitochondrial bioenergetics, insulin signaling, autophagy, and oxidative stress, which are all factors the mitochondria plays a role in. Critically, mitochondria break down across aging and heart also decrease in efficiency in aging. Key factors implicated in mitochondria morphology, such as the mitochondrial contact site and cristae organizing system (MICOS), and its role across aging remains to be seen in cardiac muscle.

Hypothesis: We hypothesized that aged cardiac muscle has alterations in mitochondria structure, which is an indication of mitochondrial dysfunction.

Methods: To better understand the relationship between mitochondria in cardiac muscle, we used transmission electron microscopy (TEM) and serial block facing-scanning electron microscopy (SBF-SEM) to quantitatively analyze the 3D networks in cardiac muscle samples of mice at aging intervals.

Results: Cardiac muscle showed some breakdown of morphological size across aging, denoted by decreased area and increased mitochondria number. In studying cristae, the inner folds of mitochondria, we observed a loss of morphology across aging. This mimicked what was observed upon CRISPR/Cas9 knockdown of Mitofilin, Chchd3, Chchd6 (some members of the MICOS complex) and Opa1 which showed poorer quality cristae and fragmented mitochondria, while mitochondria length and volume decreased.

Conclusion: We are the first to examine mitochondria changes in cardiac muscle across aging. Notably, we noticed differences in skeletal muscle, which we have previously studied, and cardiac muscle that suggests a differential response to mitochondrial aging in cardiac and skeletal muscle.

In combination, these data suggest that, in the heart, loss of the MICOS complex may be implicated in the loss of function in mitochondria that is seen across aging.

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59. High Resolution Melt Analysis Using an Internal Calibrator: Application to Tuberculosis Drug Resistance

Presenter: Logan Tsukiyama, Case Western Reserve University

Program: VUSE

Principle Investigator: Frederick Haselton, Ph.D., Department of Biomedical Engineering

Project Mentor: Nicole Malofsky, M.S., Department of Biomedical Engineering

Abstract: Drug-resistant Tuberculosis (TB) is a major global health problem for effective TB treatment. Sequence variants associated with drug resistance in TB can be detected by high resolution melt (HRM) analysis. While proven to be a rapid and highly sensitive method for genotyping and mutation screening, HRM analysis is currently limited to high-resource settings that can afford the carefully calibrated instrumentation and technical staff expertise. We are developing a robust and simple PCR design that utilizes the parallel properties of D-DNA and L-DNA enantiomers and doesn't require carefully calibrated instruments. The single-tube melt analysis method is based on each sample including L-DNA as an internal synthetic comparator with melt characteristics identical to the expected PCR product. The pre-PCR melt analysis only reflects the characteristics of the L-DNA wild-type comparator. PCR is then conducted to generate D-DNA amplicon products. A second melt analysis is performed and reflects the characteristics of the L-DNA and D-DNA. The melt characteristics of the pre-PCR and post-PCR mixtures are compared to assess the presence or absence of sequence variation in the amplicon product that may indicate drug resistance. The results of this study demonstrate the development of using L-DNA calibration in melt analysis to confirm susceptibility to isoniazid, a first-line pro-drug chemotherapy for TB, without the use of complex skills or a calibrated instrument to detect base changes associated with drug resistance in the katG gene. The advantages of this approach promise to make PCR-based genotyping simpler, more robust, and more accessible outside of well-controlled laboratory settings.

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61. Exploring Ligand Binding of Steroidogenic Factor-1 with Structural and Functional Analysis

Presenter: Andrea Mancia, Emory University

Program: MSTP Summer Research Program

Principle Investigator: Raymond Blind, Ph.D., Department of Diabetes, Endocrinology, and Metabolism

Project Mentor: Abby Örün Ph.D., Department of Diabetes, Endocrinology, and Metabolism

Additional Project Authors: Harry Choi B.S., Department of Diabetes, Endocrinology, and Metabolism

Abstract: Steroidogenic factor-1 (SF-1), a nuclear receptor, is a master regulator for endocrine function. It participates in the development of adrenal glands and gonads, and dysregulations may contribute to the development of human diseases such as endometriosis and adrenal carcinoma. Previous research has indicated that liver receptor homolog-1 (LRH-1), a closely related nuclear receptor of the same family, can bind to 15 of the 58 compounds tested in a high-throughput screen. Therefore, this project focused on identifying if various compounds from the screen would bind to the isolated ligand binding domain (LBD) of SF-1. Thus, SF-1 LBD was expressed and purified to be used for structural and functional assays. To determine ligand binding to SF-1 LBD, differential scanning fluorimetry assays were performed with compounds bilirubin, risperidone, abamectin, atenolol, PIP3, and positive control, RJW100. Of these, abamectin and RJW100 demonstrated binding to 1µM SF-1 LBD with statistically significant melting temperatures from the apo-SF-1 LBD ($p=0.0006$, $p= 0.007$). Bilirubin may have shown binding at 10µM SF-1 LBD, however, the change in melting temperature was less significant ($p=0.042$). Thus, to further evaluate, thermal melt curves were conducted to obtain IC50 values for these three compounds: 24.39µM for abamectin, and 19.93µM for RJW100, however, bilirubin could not be calculated. The next steps are to conduct fluorescence polarization assays with abamectin and RJW100 bound to coregulator peptides to obtain Kd values. Also, crystallization screens will be plated with SF-1 LBD bound to abamectin and a coregulator peptide to potentially obtain structural data of this complex.