

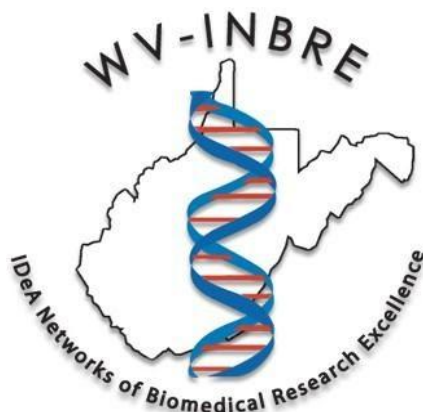
24th ANNUAL WV-INBRE
SUMMER RESEARCH SYMPOSIUM

**West Virginia IDeA Network for Biomedical Research
Excellence (WV-INBRE) Participants**

JULY 28, 2026

**West Virginia University
Morgantown, WV**

Program and Abstract Book



West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE)

24th Annual Summer Research Symposium

July 27-28, 2026

Morgantown Marriott at the Waterfront Place Hotel

Morgantown, WV

Monday, July 27

6:00 – 8:00 PM Reception Platinum Foyer (Foyer F-H)

Tuesday, July 28

8:30 - 9:00 AM Registration and Breakfast (Outside Salon ABCD)

Morning Session (Salon ABCD)

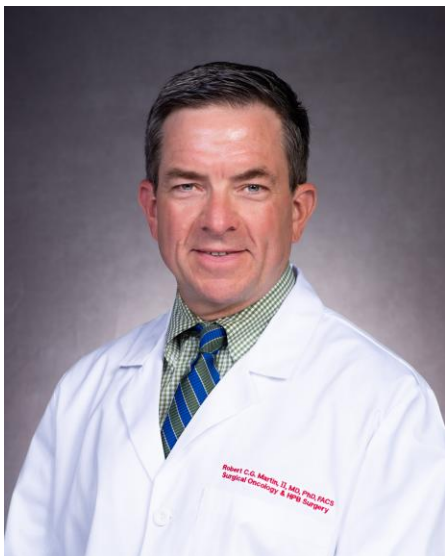
9:00 - 9:10 AM	Welcoming Remarks, Dr. Beverly Wendland , Provost, West Virginia University
9:10 - 9:20 AM	Welcoming Remarks, Dr. Ming Lei , Institutional Research Officer, West Virginia University
9:20 - 9:30 AM	Opening Remarks, Dr. Trupti Joshi , WV-INBRE Principal Investigator, Marshall University
9:30 - 9:45 AM	Breanna Waldron , WV-INBRE Summer Intern, West Virginia Wesleyan College, "Sensitizing BRCA-Proficient Triple-Negative Breast Cancer Brain Metastasis to PARP Inhibition Through Arginine Deprivation"
9:45 - 10:00 AM	Ana Chaguri , WV-INBRE Summer Intern, University of Charleston, "Identifying Genetic Factors for Obesity and Hypercholesterolemia on Chromosome 1 of TALLYHO/Jng Mice"
10:00 - 10:15 AM	Seth Perry , WV-INBRE/HSTA Fellow, Science Educator, Hurricane High School, "Ferroptosis pathway and Pterostilbene Attenuation of Hydrogen Peroxide (H ₂ O ₂) Cytotoxicity in Human Renal Proximal Tubular Epithelial (HK-2) Cells"
10:15 - 10:30 AM	Macy Hilvers , WV-INBRE Summer Intern, Glenville State University, "Characterizing the Role of NLRX1 in the Molecular Pathogenesis of COPD using a Murine Model"
10:30 - 10:45 AM	Break
10:45 - 11:00 AM	Dr. Ganga Sharma , WV-INBRE Summer Research Fellow, Assistant Professor of Physics, Fairmont State University, "Toward Precision Radiation Therapy Through Quantitative EPR Oximetry"
11:00 AM - noon	Keynote Speaker, Dr. Robert Martin , Professor and Sam and Lolita Weakley Endowed Chair, Division of Surgical Oncology, University of Louisville School of Medicine, Louisville, KY "Advances in the Surgical Care of Pancreatic and Hepatobiliary Malignancies: A Career Journey Through Innovation, Research, and Mentorship"

Noon - 1:00 PM **Recognition and Networking Lunch (Salon ABCD)**

Afternoon Session

1:00 - 2:00 PM Career Panel (Salon ABCD)

2:00 - 3:30 PM Poster Session (Salon EFGH)



"Advances in the Surgical Care of Pancreatic and Hepatobiliary Malignancies: A Career Journey Through Innovation, Research, and Mentorship"

Dr. Martin is a Professor and the Sam and Lolita Weakley Endowed Chair in the Division of Surgical Oncology at the University of Louisville School of Medicine. He also serves as Director of the Surgical Oncology Program. As a surgical oncologist with fellowship training in hepatobiliary surgery at Memorial Sloan-Kettering Cancer Center and a PhD in Pharmacology and Toxicology, Dr. Martin's career has centered on integrating clinical oncology, translational science, and innovative therapeutic development.

Dr. Martin's research program focuses on advancing the treatment of hepatobiliary, pancreatic, and foregut malignancies through three interconnected themes: (1) developing and optimizing ablative technologies such as irreversible electroporation (IRE); (2) improving combination strategies that pair local ablation with systemic immunotherapy; and (3) elucidating the molecular, immunologic, and epigenetic mechanisms that drive aggressive and treatment-resistant cancers. Dr. Martin's clinical and translational work has helped define the role of IRE in locally advanced pancreatic cancer (LAPC). He led one of the earliest Phase 1b trials investigating the combination of IRE with immunotherapy and he has published extensively on IRE's safety profile, technical parameters, and survival benefits. Recent work has identified margin accentuation with intraoperative IRE as a strategy to reduce local recurrence following pancreatic cancer resection. Parallel investigations demonstrate that IRE enhances intratumoral drug delivery, improves chemotherapy concentration, and creates a favorable microenvironment for subsequent immunologic activation.

Dr. Martin has served as PI or co-investigator on multiple federally funded and industry-supported projects. He also leads an investigator-initiated clinical program evaluating immunotherapy and IRE for advanced pancreatic cancer and exploring the addition of adjuvant beta-glucan in LAPC. As a long-standing mentor in the NIH R25 Cancer Education Program, Dr. Martin has supported the training of clinician-scientists across surgical oncology and cancer biology.

Dr. Martin's scientific contributions span more than 200 peer-reviewed publications, advancing knowledge of liver, pancreatic, esophageal, gastric, colorectal cancers, bile duct tumors, sarcoma, and melanoma. Early in his career, he demonstrated the importance of timely diagnosis and systematic management in foregut malignancies. His basic science efforts have emphasized epigenetic drivers of pancreatic cancer progression and the mechanisms by which ablative therapy modulates anti-tumor immunity. Recent studies show that epigenetic modulation can significantly enhance the efficacy of immunotherapeutic strategies in pancreatic cancer, while intratumoral immunotherapy paired with ablation can produce robust systemic responses.

Dr. Martin's research aims to transform local and systemic cancer therapy by uniting advanced surgical technologies with mechanistic biological insights, ultimately improving survival and quality of life for patients with some of the most lethal malignancies.

POSTER PRESENTATION ASSIGNMENTS

Please note your poster board assignment number. You should be available at your poster during the poster session.

Abstracts on the following pages appear in the order of these poster board assignments.

1. Plasma-Derived Large Extracellular Vesicles Promote Cerebrovascular Endothelial Dysfunction in IBD. Mariam S. Al-Zoubi¹, Erika Butcher², Ji Bihl², Subha Arthur².¹Department of Exercise Science, West Virginia Wesleyan College, Buckhannon, WV. ²Department of Biomedical Sciences, Marshall University Joan C. Edwards School of Medicine, Huntington, WV.

2. The Effect of Ganoderic Acids from Reishi Mushroom (*Ganoderma lucidum*) on Cell Proliferation, Migration and Matrix Metalloproteinases in Breast Cancer. Madelyn E. Armstrong, Dane V. Heath, Barrett A. McCain, Lanna K. Nguyen, Megan D. Brozik, Caden L. Carpenter, Colten R. Workman and Luke G. Huggins. Department of Biology and Environmental Science, West Virginia Wesleyan College, Buckhannon, WV.

3. Building Metastatic Microenvironments Through Enzyme Filamentation. Nicholas S. Asher[†], Heather L. Hansen*, Bradley A. Webb*. [†]Department of Natural Sciences, Fairmont State University, Fairmont, WV. *Department of Biochemistry and Molecular Medicine, WVU School of Medicine, Morgantown, WV.

4. Investigating the Effects of Synthetic Vape Coolants on Nicotine Reward-Related Behaviors. Cameron Bailey, Brandon Henderson, Ph.D., Marshall University, Huntington, WV.

5. Investigating the Role of ERO1A in Murine Behavior Aaron J. Barbee*, Santhosh Shanthi Bhupathi*, Terri J. Poling[†], James C. Walton[†], Lori A. Hazlehurst*. *Cancer Institute, West Virginia University, Morgantown, WV [†]Department of Neuroscience, School of Medicine, West Virginia University, Morgantown WV

6. Evaluation of the Antimicrobial Activity of Typhinamycin against Pathogenic Bacteria. Amelia F. Bell*, Joseph Horzempa*. *Department of Biological Sciences, West Liberty University, West Liberty, WV

7. Spatial Distancing on Solid Media Determines Horizontal Gene Transfer of Plasmid DNA in *Pseudomonas aeruginosa*. Jaden B. Bender¹, Aimee M. Iradukunda², Noah J. Sauve², Newton Neog², Mackenzie A. Hall¹, Hayden T. Hess², Joseph Horzempa¹, and Yan-Yeung Luk², 1. Department of Biological Sciences, West Liberty University, West Liberty, West Virginia 2. Department of Chemistry, Syracuse University, NY.

8. Synthesis, Structural Characterization, and In Vitro Antiproliferative Activity of Indole-Coumarin Hybrids against Breast Cancer Cell Lines. Rylyn A. Bielinski¹, Maryangeli Calderón-Sánchez², Zulma Ramos², and Eliud Hernández-O'Farrill². ¹West Liberty University, West Liberty, WV. ²University of Puerto Rico, Medical Sciences Campus, School of Pharmacy, Department of Pharmaceutical Sciences

9. Targeted Mutation of FTL_1711 in *Francisella tularensis* Live Vaccine Strain. Audrey Blust* and Joseph Horzempa*. *Department of Biological Sciences, West Liberty University, West Liberty, WV.

10. Genetic Polymorphism in Lipid Droplet Protein CIDEC and Obesity in Mice Jayden Cadle, Ana Chaguri, Jung Han Kim Department of Biomedical Sciences, School of Medicine, Marshall University, Huntington, WV.

11. Identifying Genetic Factors for Obesity and Hypercholesterolemia on Chromosome 1 of TALLYHO/JngJ Mice. Ana Chaguri, Jayden Cadle, Jung Han Kim. Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University

12. The Na⁺/K⁺-ATPase α 1 Signaling Pathway in Adverse Cardiac Remodeling Vinu Chandrasekar¹, Sidney Strause², Karleigh Burdette², Marco T. Pessoa², Liquan Cai², and Sandrine V. Pierre² ¹Marshall University, Huntington, WV ²Joan C. Edwards School of Medicine, Department of Biomedical Sciences, Huntington, WV

13. The role of chemokines in habenular axon outgrowth in zebrafish. Dakota Charles*, Gabrielle Justice*, Anthony Poe*, Din Rayche*, Wilthin Alcime*, Sara Roberson*. *Department of Applied Science, Bluefield State University, Bluefield WV.

14. Anatomical Mapping and Functional Imaging of Stress-Responsive Modulation of Sensory Processing. Manuella Cogliatti†, Lindsey Moraes*, Ph.D. Ramiro Comas Mutis*, Ph.D. Morgan Bridi*, †-Department of Biology and Environmental Science, Davis and Elkins College, Elkins, WV *Department of Neuroscience, West Virginia University, Morgantown, WV.

15. Antimicrobial Activity of *Verbascum thapsus* Essential Oil Nanoemulsion Against Drug-Resistant Bacteria Jonathan Cooper, Mukesh Sivakumaran, and Shreyas Varathan West Virginia School of Osteopathic Medicine, Lewisburg, WV.

16. Bidirectional, Sex-Dependent Effects of Rosiglitazone on Astrocyte–Synapse Remodeling and Behavior Following Adolescent Binge Ethanol Exposure Kadence Crank¹, Darshan Sangani¹, Tristan Muck¹, Olivia Coulter¹, Mika Jones^{1,2}, Hannah Sexton^{1,2}, Mary-Louise Risher^{1,2} ¹Department of Biomedical Research, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25701, USA ²Neurobiology Research Laboratory, Hershel “Woody” Williams Veterans Affairs Medical Center, Huntington, WV 25704, USA

17. Kaempferol reduces cytotoxicity of hydrogen peroxide and cisplatin in renal proximal tubule cells Kiersten Davis+, Christopher Bender*, Katie Brown*, Elizabeth McGuffey*, Monica Valentovic* *Department of Biomedical Sciences, Toxicology Research Cluster, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV;+Fairmont State University, Fairmont, WV

18. The *Pseudomonas aeruginosa* 1244 pilin glycan enhances twitching motility and renders bacteria more susceptible to human beta defensin 2. *Shania Davis & Joseph Horzempa, Department of Biological Sciences, West Liberty University, West Liberty, WV USA.*

19. Investigating NDRG1 and Rb Interactions in Clear Cell Renal Cell Carcinoma. *Ashton Dean¹, Chelsea McCallister², Sam Roth², Travis Salisbury². ¹University of Charleston, Charleston, WV. ²Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, 1 John Marshall Drive, Huntington, WV*

20. Defining Adolescent Tripartite Synapse Maturation Using Integrated Spatial Transcriptomics and Stimulated Emission Depletion (STED) Microscopy *Claire Denning¹, Nisha Kumar¹, Mika Jones¹, Aaliyah Walls¹, Grace Angelona¹, Tristan Muck¹, Faith Malone¹, Olivia Coulter¹, Hannah Sexton^{1,2}, Krista Denning³, Hadi Ul Bashar⁴, Sadia Akter¹, Mary-Louise Risher^{1,2}. ¹Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25701, USA; ²Neurobiology Research Laboratory, Hershel 'Woody' Williams Veterans Affairs Medical Center, Huntington, WV 25704, USA; ³Department of Pathology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25701, USA; ⁴Department of Biological Sciences, Marshall University, Huntington, WV 25755, USA*

21. Active Hexose Correlated Compound Enhances Macrophage Stimulation in Stressed Mice during *Chlamydia muridarum* Genital Infection. *Isabella Dibbisio, Gebreegziabher Belay, Tesfaye Belay. Bluefield State University, Bluefield, WV.*

22. Optimization of Acetalated Dextran Nanoparticles for Targeted Drug Delivery. *Kaitlynn Drainer, Rukiye Nurseker, Sharan Bobbala. *Department of Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Morgantown, WV*

23. Sex-Dependent Modulation of Induced-Thyrotoxicosis in Developing Chicken Embryos *Logan Eddy, Nicole Garrison, and Holly Vopal* Department of Biological Sciences, West Liberty University, West Liberty, WV

24. Expression profiling of soleus muscle in TALLYHO and C57BL/6 mice. *Sophia Ellingson, West Virginia State University; Dr. James Denvir, Marshall University; Dr. Jung Han Kim, Marshall University; Kristiana Skiffington, Marshall University*

25. Biotherapeutic Potential of Bacterial Species Living on Plants in Southern West Virginia. *Sydney Feazell, OMS-II, Matthew Williams PhD West Virginia School of Osteopathic Medicine*

26. Nephrotoxicity and the Mechanism of 2,6-Dichlorohydroquinone in Isolated Rat Kidney Cells. *Maeghan B. Fisher, Cade Cole, Mika McCormick, Dianne K. Anestis, Gary O. Rankin, Department of Biomedical Sciences, Marshall University, Huntington, WV, Department of Science and Technology, Fairmont State University, Fairmont, WV.*

27. Impact of Early Life Stress and Gestational Stress on Relevant Behaviors in Pregnant Mice: Implications for Peripartum Mental Health Disorders *Makel E. Galloway, Marissa M. Nicodemus, Kathleen E. Morrison* Department of Psychology, West Virginia University

28. Assessment of Movement and Motivation Deficits in C57BL/6J Mice Before and After Photothrombotic Stroke: Towards an Ensemble-Based Strategy for Improving Post-Stroke Recovery Maggie Gunter^{1,3}, Ekaterina Weil, PhD¹; Vincent Setola, PhD^{1,2} ¹Department of Neuroscience, ²Department of Behavioral Medicine and Psychiatry, West Virginia University School of Medicine Health Sciences Center, Morgantown, WV; ³West Liberty University Center for Biomedical Research, Wheeling, WV

29. Development of a Subarachnoid Hemorrhage Mouse Model to Study Artificial Light at Night on Patient Outcomes. Madalyn Harless^{1,2}, Tara K.S. Craft¹, Terri J. Poling¹, James C. Walton¹, Kate Karelina¹, A. Courtney DeVries^{1,3}, Randy J. Nelson¹. ¹School of Public Health, ²Department of Neuroscience, Rockefeller Neuroscience Institute, ³Department of Medicine, West Virginia University, PO Box 9303, Morgantown WV, 26506, USA

30. Investigating Appalachian Plants as a Source of Next-Generation Biotherapeutics Kyle Hawthorne, Matthew Williams

31. Establishment of an Avian Model of Induced Thyrotoxicosis and Its Effects on Cranial Suture Osteogenesis. Tyler P. Hill, Holly L. Vopal. Department of Biological Sciences, Hilltop Center for Biomedical Research, West Liberty University, West Liberty, WV.

32. Role of NOD-Like Receptor X-1 in Chronic Obstructive Pulmonary Disease Macy Hilvers¹, Madison Cooper¹, Yulu Li¹, Salik Hussain¹ ¹Physiology, Pharmacology and Toxicology, School of Medicine, West Virginia University, Morgantown, WV

33. Biodistribution and Cardiac Safety Evaluation in Severely Immunosuppressed Mice After Intramuscular Injection of Human Myogenic Progenitor Cells. Munachi Ibeawuchi, John Crutchley*, Marco T. Pessoa*, Liquan Cai*, Kailey Stuart*, Sandrine V. Pierre*. *Department of Biomedical Sciences, Marshall University, Huntington, WV.

34. Testing the Effect of Different Doses of Active Hexose Correlated Compound (AHCC) on McCoy Cell Growth during *Chlamydia muridarum* Infection. Gabrielle Justice, Gebreegzabher Belay, Tesfaye Belay. Bluefield State University, Bluefield, WV.

35. The Role of Dorsal Root Ganglion Macrophages in Regulating the Sensations of Touch and Heat. Samantha I. Keith*†, Wen Zheng*, Terri Poling‡, Kody M. Moore*, Edwin Wan*. *Department of Microbiology, Immunology and Cell Biology, West Virginia University School of Medicine, Morgantown WV, †Shepherd University, Shepherdstown WV, ‡Rodent Behavior Core, West Virginia University School of Medicine, Morgantown WV

36. Isolation and Characterization of Potential Live Biotherapeutic Products from the Greenbrier River Against Common Bacterial Pathogens. Sofia Kelso, Dr. Matthew Williams

37. Preventing Mitochondrial Dysfunction in Aging Payton Leary*, Lawrence Grover*, PhD, Werner Geldenhuys[^], PhD, Nalini Santanam, PhD, MPH* *Department of Biomedical Sciences, Marshall University, Huntington, West Virginia, [^]WVU School of Pharmacy, Morgantown, WV.

38. Characterizing the effects of NL-1 treatment on induced ferroptosis in SIM-A9 cells. Maddie Loughborough^{1,2}, Enrique M. Carrera^{1,3}, Werner Geldenhuys^{1,3}, Jason D. Huber^{1,3}. ¹West Virginia University School of Medicine, West Virginia University, WV; ²Concord University, Athens, WV; ³Department of Pharmaceutical Sciences, West Virginia University, WV.

39. Phytochemical from *Rhus copallinum* extract exhibits antimicrobial activity toward ESKAPE pathogens. Kathleen Loughman*†, Claudia Ospina^Δ, and Joseph Horzempa*. *Department of Biological Sciences, West Liberty University, West Liberty, WV; †John Marshall High School, Glen Dale, WV; Department of Natural Sciences and Mathematics, ^ΔInter American University of Puerto Rico, Bayamón, PR

40. Evaluation of Protein Refolding in Type 2 Diabetic Heart. Gilbeiry Marte Diaz, Comfort Olowu. Remi Nohoesu, Atefeh Razazan, Hafsat Alabere, Joshua Moug, Mark Eminhizer, John Hollander. Division of Exercise Physiology, Mitochondria, Metabolism, & Bioenergetics, Department of Physiology, Pharmacology, & Toxicology. West Virginia University.

41. HERMES-AI: An Interpretable AI Framework for Modeling Immune Pathway Instability During Long-Duration Human Spaceflight. Michael J. Martínez Vega¹², Mohammad Vahed, PhD³⁴, Trupti Joshi, PhD³⁵. ¹Department of Biology, University of Puerto Rico at Humacao, Humacao, PR; ²University of Puerto Rico Comprehensive Cancer Center, San Juan, PR; ³Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV; ⁴West Virginia IDeA Network of Biomedical Research Excellence, Marshall University, Huntington, WV; ⁵Translational Bioinformatics and AI Innovations Lab, Marshall University, Huntington, WV

42. Impact of Norepinephrine on the Gene Expression of Siderophore Genes in Starved and Non-starved *Pseudomonas aeruginosa* Isolates. Michelle Martinez¹, Emily Shupe³. Gebreegziabher Belay², Tesfaye Belay². ¹Bluefield Middle School, Bluefield, WV, ²Bluefield State University, Bluefield, WV, ³West Virginia School of Osteopathic Medicine, Lewisburg, WV.

43. Antibacterial effects of *Monarda Fistulosa* (Bee Balm) nanoemulsion against drug-resistant bacterial pathogens. Miguel Mascarenhas, Madhav Nair, and James Wilkes West Virginia School of Osteopathic Medicine, Lewisburg, WV

44. Caffeine Effects on Locomotor Activity of Madagascar Hissing Cockroaches. Isadora Matthews*, Katharine Birkman*, Ruth Conley*, Qing Wang#. *Department of Natural and Physical Sciences, Shepherd University, Shepherdstown, WV; #Department of Computer Science, Mathematics and Engineering, Shepherd University, Shepherdstown, WV.

45. Restoration of Immune Responses in a beta2-Adrenergic Receptor-Deficient Stress Murine Model during *Chlamydia muridarum* Genital Tract Infection. Robert McClain², Gebreegziabher Belay¹ Tesfaye Belay³. ¹Bluefield State University, Bluefield, WV ²Woodrow High School, Beckley, WV.

46. KAP1 Cooperates with HIF Signalling to Regulate Hypoxia-Driven Gene Expression in Breast Cancer. Millar McElrea, Dr Alexey Ivanov. Department of Biology-Chemistry, University of Charleston, Charleston, WV; Department of Biochemistry and Molecular Medicine, West Virginia University School of Medicine, Morgantown, WV.

47. Effects of Exercise and Vaping on Adolescent Behavior. Katherine Meek, Nick Williams, Elaina Blickenstaff, Samuel Tetteh-Quarshie, Lawrence Grover, Brandon Henderson, Nalini Santanam. Department of Biomedical Sciences, Marshall University Joan C. Edwards School of Medicine, Huntington, WV.

48. Assessing Caffeine Preference and Addiction-Like Feeding Behavior in Madagascar Hissing Cockroaches (*Gromphadorhina portentosa*) Using a Two-Choice Preference Arena. Juan J. Mercado-Trinidad*, Ruth Conley**, Qing Wang#. *Spring Mills High School, Martinsburg, WV; **Department of Natural and Physical Sciences, Shepherd University, Shepherdstown, WV; #Department of Computer Science, Mathematics and Engineering, Shepherd University, Shepherdstown, WV

49. Selection of *Neisseria gonorrhoeae* mutants resistant to resorufin morpholinoethyl ether. Rebekkah Merinar¹, Alex Tarr¹, Mackenzie Hall¹, Randall A. Koziel², Vahid Lotfikalajahi², Brian V. Popp², Hacer Karatas Bristow², and Deanna M. Schmitt¹, ¹Department of Biological Sciences, West Liberty University, West Liberty, WV; ²C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV

50. Effects of Macromolecular Crowding on the Kinetics of Pyruvate Kinase Muscle 1 with Variable ADP and PEP Substrates Jannah N. Miller, Rafe M. Riley, Kelsey A. Aldrich Department of Chemistry and Biochemistry, West Virginia Wesleyan College, Buckhannon, West Virginia

51. Synovium-Associated Stem Cells: Comparative Biology of Synovium-Derived and Infrapatellar Fat Pad-Derived Stem Cells as Superior Sources for Cartilage Regeneration. Catherine A. Milliman†, Ming Pei*. † West Virginia IDeA Network for Biomedical Research Excellence, Sumer Research Program, Morgantown, West Virginia; Tissue Engineering Laboratory, Division of Exercise Physiology, Department of Orthopedics, West Virginia University, Morgantown, West Virginia

52. Increased Keratin 18 Expression Suppresses Brain Metastatic Potential of Breast Cancer Cells. Abigail Mills, OMS II ¹; William Liu, OMS II ¹; Maggie Maguire, OMS II ¹; David Brooks, OMS II ¹; Christopher Butler, BS ¹; Gabor Szalai, PhD ²; Anissa Johnson, DO ³; Garrett Clemons, PhD ¹; Travis Salisbury, PhD ⁴; James Denvir, PhD ⁴; Paul Lockman, PhD ⁵; Tuoan Liu, MD, PhD ¹. 1. Department of Biomedical Sciences, West Virginia School of Osteopathic Medicine, Lewisburg, West Virginia, United States of America. 2. Department of Biomedical Sciences, Burrell College of Osteopathic Medicine, Las Cruces, New Mexico, United States of America. 3. School of Medicine, Loma Linda University, Loma Linda, California, United States of America. 4. Genomics and Bioinformatics Core Facilities, Marshall University, Huntington, West Virginia, United States of America. 5. Department of Pharmaceutical Sciences, College of Pharmacy, West Virginia University, Morgantown, West Virginia, United States of America.

53. Monocarboxylate Transporter 2 (MCT2) Mitigates Lung Tumor Progression by Shaping the Immune Microenvironment Duyen Nguyen ¹, Yen On Chan ^{2,3}, Zhuanhong Qiao ⁴, Trupti Joshi ^{2,3,5,6}, David Gozal ⁴ and Abdelnaby Khalyfa ²*Department of Biomedical Sciences, University of Charleston, Charleston, WV 25304. Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, 25755. Christopher S Bond Life Science Center, University of Missouri-Columbia, MO, 65201. Department of Pediatrics, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, 25755. MU Institute for Data Science and Informatics, University of Missouri-Columbia, MO, 65201. Department of Biomedical Informatics, Biostatistics, and Medical Epidemiology, School of Medicine, University of Missouri-Columbia, MO, 65201

54. Na⁺/K⁺-ATPase Modulation with Ouabain Induces Protection of Human Induced Pluripotent Stem Cell-Derived Cardiac Progenitor Cells Against Hypoxia: Role of Metabolic Preconditioning. Zackary D. Nunley¹, Sandrine V. Pierre^{2,3}, Marco T. Pessoa^{2,3}.¹Department of Chemistry, Marshall University, Huntington, WV; ²Department of Biomedical Sciences, Marshall University, Huntington, WV; ³Joan C. Edwards School of Medicine, Huntington, WV.

- 55. Investigating the Effects of NL-1 on Induced Ferroptosis in IMA2.1 Cell Line** *Ridwan Oloyede^{1,2}, Enrique M. Carrera^{1,3}, Dr. Werner Geldenhuys^{1,3}, Jason D. Huber^{1,3}. ¹West Virginia University School of Medicine, West Virginia University, WV; ²Glenville State University, Glenville, WV; ³Department of Pharmaceutical Sciences, West Virginia University, WV.*
- 56. Characterizing the Contribution of Histamine on Cardiomyocyte Hypertrophy.** *Marin Parker, Heather Linsky, Kallie Shaffner, Alexander Widiapradja, Scott Levick.*
- 57. Putting the “Pro” in Probiotics: How Microorganisms are Changing the Playbook** *Sagar S. Patel, MBS, OMS-II*, Matthew Williams, PhD*. West Virginia School of Osteopathic Medicine, Lewisburg, WV*
- 58. Ferroptosis pathway and Pterostilbene Attenuation of Hydrogen Peroxide (H₂O₂) Cytotoxicity in Human Renal Proximal Tubular Epithelial (HK-2) Cells.** *Seth Perry#, Kathleen C. Brown*, Elizabeth McGuffey*, Christopher Bender* and Monica Valentovic*. #Hurricane High School, Hurricane, WV; *Department of Biomedical Sciences, Toxicology Research Cluster, Joan C. Edwards School of Medicine Marshall University, Huntington, WV.*
- 59. Structure-Activity Relationship (SAR) studies identify DOHEVANIL as a potent, non-pungent growth-suppressive agent in human adenocarcinoma (ASC-L).** *Joeusel A. Ramos Torres¹, Aliyah N. Webb², Sarah D. Wooten³, Sarah L. Miles³, and Piyali Dasgupta³. ¹University of Puerto Rico at Mayagüez, ²MTTC, ³Department of Biomedical Sciences, Marshall University, Huntington, WV*
- 60. Localization of the divisome protein FtsL in Francisella tularensis LVS.** *Abigale Riggle and Stuart Cantlay, Department of Biological Sciences, West Liberty University, West Liberty, WV, 26074.*
- 61. Investigating Myogenic Regulation of Craniofacial Morphology in a Avian Model of Craniosynostosis.** *Olivia M. Rocchio, Emily K. Stotts, Tyler P. Hill, Dr. Nicole Garrison, and Dr. Holly L. Vopal. Department of Biological Sciences, Hilltop Center for Biomedical Research, West Liberty University, West Liberty, WV*
- 62. The Effects of Vaping on Liver extracellular vesicles from adolescent mice.** *John Rose, Jadyn Simon, Samuel Tetteh-Quarshie, Brandon Henderson, and Nalini Santanam. Department of Biomedical Sciences, Marshall University, Huntington, West Virginia.*
- 63. Active Hexose Correlated Compound Enhances Dendritic Cell Stimulation in Stressed Mice during Chlamydia muridarum Genital Infection.** *Jaida Rottenbery, Gebreegziabher Belay Tesfaye Belay. Bluefield State University, Bluefield, WV.*
- 64. Establishment of Human iPSC-Derived Skeletal Muscle Cells to Investigate Na⁺/K⁺-ATPase Signaling During Muscle Regeneration.** *James A. Scott, Sandrine V. Pierre, Liquan Cai, Department of Biomedical Sciences; Joan C. Edwards School of Medicine, Marshall University, Huntington, WV*
- 65. Toward Precision Radiation Therapy Through Quantitative EPR Oximetry** *Ganga P. Sharma¹, Benoit Driesschaert^{2,3}. ¹Department of Natural Sciences, Fairmont State University ²WVU Cancer Institute Research Programs, WVU Cancer Institute ³Department of Pharmaceutical Sciences, WVU School of Pharmacy*
- 66. A Genetic Rescue Model to Investigate the Therapeutic Window for Degenerating Cones** *Avery Simth, Brooke Bothers, Madyson Ashcraft, Wen-Tao Deng. Department of Ophthalmology and Visual Sciences, Department of Biochemistry and Molecular Medicine, West Virginia University, Morgantown, WV 26506*

- 67. Thyroxine Exposure Enhances Late Stage Osteoblast Maturation in Cranial Sutures From Embryonic Chickens Using an Avian Model of Induced Thyrotoxicosis.** *Emily K. Stotts, Olivia M. Rocchio, Tyler P. Hill, Dr. Holly L. Vopal. Department of Biological Sciences, Hilltop Center for Biomedical Research, West Liberty University.*
- 68. The Effects of Circulating Extracellular Vesicles on Endothelial Progenitor Cell Function Under Hypertensive Conditions.** *Madison Stratton, Shuzhen Chen, Jinju Wang. Department of Biomedical Sciences, John C. Edwards School of Medicine, Huntington, WV*
- 69. Pyruvate Kinase L is Sensitive to Physiological Macromolecule Concentrations.** *Addison P. Taylor, Mary L. Genes, Kelsey A. Aldrich. Department of Chemistry and Biochemistry, West Virginia Wesleyan College, Buckhannon, West Virginia*
- 70. Early Neurobehavioral Effects of Prenatal Nano-Titanium Dioxide Exposure in Adult Sprague-Dawley Rats.** *Sabrina Thorn¹, Teresa Gluth¹, Taryn Veltri¹, Landen Bach¹, Sarah Keeley¹, Jacob W. Feldmann¹, Terri Poling², James Walton², Elizabeth C. Bowdridge¹. West Virginia University Department of Physiology, Pharmacology, & Toxicology and Department of Neuroscience.*
- 71. Comparison of two pre-clinical AAV gene therapy vectors in mouse models of blue cone monochromacy.** *Mary E. Tracey, Brooke A. Brothers, Madison Ashcraft, Qingyan Wang, Saya Geisendorfer, Wen-Tao Deng. Dept. of Ophthalmology / Dept. of Biochemistry & Molecular Medicine, West Virginia University.*
- 72. A Method for Extracting and Purifying and Modifying, 1 mg Quantities of Sialylglycopeptide for Capillary Electrophoresis Assays.** *Emerson Tracy¹, Tolulope Betty Akingbaso², Eric Corley², Daniel Oladuga², Lisa Holland². ¹Department of Shepherd University, Shepherdstown, WV. ²C. Eugene Bennett Chemistry Department, West Virginia University, Morgantown.*
- 73. Differences in DNA Methylation Profiles Associated with HPV Status Suggest Disruptions in Cancer Associated Pathways.** *Shayna M. Tysor¹, Juliana M. Serrano-Rodríguez^{2,3}, Jurelis Torres-Reyes^{2,4}, Jeannette L. Salgado-Montilla², Gabriel Borges-Vélez², and Josué Pérez-Santiago^{2,4}
¹Shepherd University, Shepherdstown, West Virginia ²University of Puerto Rico Comprehensive Cancer Center, San Juan, Puerto Rico ³University of Puerto Rico Rio Piedras Campus, San Juan, Puerto Rico ⁴University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico*
- 74. Sex-Specific Cognitive Effects of Prenatal Nano-TiO₂ Exposure and Preventive Febuxostat Treatment.** *Taryn G. Veltri*, Teresa D. Gluth†, Sabrina L. Thorn†, Terri J. Poling†, Elizabeth C. Bowdridge†. *Department of Biology and Environmental Sciences, West Virginia Wesleyan College, WV; †Department of Physiology, Pharmacology, and Toxicology, West Virginia University, WV*
- 75. Sensitizing BRCA-Proficient Triple-Negative Breast Cancer Brain Metastasis to PARP Inhibition Through Arginine Deprivation** *Breanna Waldron¹, Olivia Carpenter-Milam¹, Cullen Wolford¹, Geoffrey Pecar^{1,2}, Paul R. Lockman¹ Department of Basic Pharmaceutical Sciences, School of Pharmacy, West Virginia University (HSC), 64 Medical Center Dr., Morgantown, WV 26505 MD/PhD Program, School of Medicine, West Virginia University (HSC), 64 Medical Center Dr., Morgantown, WV 26505*
- 76. Inhibition of SARS-CoV-2 3CL protease (NSP5) Activity with mucosal IgA antibodies** *Taytum Walls, Meenal Elliott, Timmy Nguyen*

77. The non-pungent capsaicin analog DOHEVANIL triggers ferroptosis in human endometroid ovarian carcinoma (EOC) cells. Aliyah N. Webb¹, Joeusel Torres Ramos², Sarah D. Wooten³, Sarah L. Miles³ and Piyali Dasgupta³. ¹BridgeValley Community, and Technical College, Charleston, WV, ²University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico, ³Department of Biomedical Sciences, Marshall University, Huntington, WV

78. Endometriosis and Cardiovascular Health: An understudied connection. Samiya Webb, Jadyn Simon, Harshal Sawant. Brenda Mitchell, MD, Nalini Santanam, PhD, MPH

79. Genetic Differentiation of Nutritionally Deprived *Pseudomonas aeruginosa*. Daron Weekley*, Tesfaye Belay[†] and Stuart Cantlay*. *Dept of Biomedical Sciences, West Liberty University, WV, 26074. [†] Bluefield State University, Bluefield, W.V. 24701.

80. Fate-mapping Pseudohypoxic Cells in Triple-Negative Breast Cancer Hannah Wheaton*, Elena Pugacheva[†] *Department of Natural and Physical Sciences, Shepherd University, Shepherdstown WV [†]WVU Cancer Institute, West Virginia University, Morgantown WV

81. Determining Gene Expression Changes Induced by Ganoderic Acid A in Estrogen Receptor Positive and Estrogen Receptor Negative Human Breast Cancer Cells. Claire Wheelert[†], Travis Salisbury*, Mohit Verma*. *Marshall University Joan C. Edwards School of Medicine, Huntington, WV; [†]West Virginia Wesleyan College, Buckhannon, WV

82. Development of novel biotherapeutics for the treatment of opportunistic pathogens in humans. Garon Wiseman, Matthew Williams

83. Reprogramming of Branched-Chain Amino Acid Metabolism in Breast Cancer Nevaeh Ann Ellington, Emine C. Koc, Hasan Koc

ABSTRACTS

1. Plasma-Derived Large Extracellular Vesicles Promote Cerebrovascular Endothelial Dysfunction in IBD. *Mariam S. Al-Zoubi¹, Erika Butcher², Ji Bih², Subha Arthur².*¹*Department of Exercise Science, West Virginia Wesleyan College, Buckhannon, WV.* ²*Department of Biomedical Sciences, Marshall University Joan C. Edwards School of Medicine, Huntington, WV.*

Inflammatory bowel disease (IBD) is increasingly recognized as a systemic inflammatory disorder associated with numerous extraintestinal complications. Epidemiological studies report a 15-30% increased risk of cerebrovascular complications, including ischemic stroke, in IBD patients, even in the absence of traditional vascular risk factors. However, the mechanisms linking intestinal inflammation to cerebrovascular injury remain poorly understood. Extracellular vesicles (EVs), circulating mediators of intercellular communication, transport inflammatory cargo capable of influencing vascular function at distant sites. We hypothesized that plasma-derived large EVs from patients with IBD promote cerebrovascular endothelial dysfunction by inducing endothelial activation and disrupting barrier integrity. To test this hypothesis, human brain microvascular endothelial cells (HBMECs) were exposed for 24 hours to plasma-derived large EVs isolated from patients with IBD. Protein expression of endothelial activation markers and tight junction proteins was assessed by Western blot analysis. Treatment with IBD-derived EVs significantly increased expression of intercellular adhesion molecule-1 (ICAM1; n=4, p<0.05) and vascular cell adhesion molecule-1 (VCAM1; n=4, p<0.05), indicating enhanced endothelial activation. In contrast, expression of the tight junction proteins Claudin-5 and zonula occludens-1 (ZO-1) was reduced (n=4, p<0.05), consistent with impaired barrier integrity and increased endothelial permeability. These findings demonstrate that circulating EVs from patients with IBD directly induce a pro-inflammatory and barrier-disruptive phenotype in cerebrovascular endothelial cells. Collectively, our results identify EV-mediated endothelial dysfunction as a potential mechanistic link between intestinal inflammation and elevated stroke risk in IBD and suggest that circulating EVs may represent novel biomarkers and therapeutic targets for prevention of cerebrovascular complications in IBD patients. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence).***

2. The Effect of Ganoderic Acids from Reishi Mushroom (*Ganoderma lucidum*) on Cell Proliferation, Migration and Matrix Metalloproteinases in Breast Cancer. *Madelyn E. Armstrong, Dane V. Heath, Barrett A. McCain, Lanna K. Nguyen, Megan D. Brozik, Caden L. Carpenter, Colten R. Workman and Luke G. Huggins. Department of Biology and Environmental Science, West Virginia Wesleyan College, Buckhannon, WV.*

*Breast cancer accounts for approximately 30% of all reported cancer cases in females in the US. Both triple-negative breast cancer (TNBC) and Luminal A are common subtypes that can be challenging to treat with current anti-cancer therapies. The Reishi mushroom (*Ganoderma lucidum*) produces a set of bioactive triterpenoids, the Ganoderic Acids (GAs) that are thought to be the main active ingredients underlying the anti-cancer activities in *G. lucidum* extracts. Here, we examine the effects of *G. lucidum* extract (GLE) and Ganoderic Acids A, B and D (GAA, GAB, and GAD, respectively) on the TNBC cell line MDA-MB-231, and Luminal A breast cancer cells lines MCF-7 and T-47D as well as the non-cancer line MCF 10A. GLE significantly inhibited the proliferation of MDA-MB-231 TNBC cells, but had no effect on the Luminal A MCF-7 and T-47D cells. GAA inhibited the proliferation of the TNBC MDA-MB-231 cells, but not the Luminal A MCF-7 nor T-47D. GAB did not inhibit the proliferation of MDA-MB-231, MCF-7, or T-47D cells. GAD significantly inhibited the Luminal A lines, but had no effect on the TNBC MDA-MB-231 cells. Neither GLE, nor the Ganoderic Acids appear to inhibit the proliferation of the normal MCF 10A line. GLE significantly inhibited the migration of MDA-MB-231 TNBC cells, but neither GAA, GAB, nor GAD had a significant effect. In addition, we have examined the expression and activity of the Matrix Metalloproteinase MMP-2 in each cell line in response to GLE and the GAs. (Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence, Grants to Dr Joanna Webb (WVWC) from the West Virginia Higher Education Policy Commission Division of Science and Research, the Maier Foundation, and the McCuskey Family Fellowship)*

3. Building Metastatic Microenvironments Through Enzyme Filamentation. Nicholas S. Asher[†], Heather L. Hansen^{*}, Bradley A. Webb^{*}. [†]*Department of Natural Sciences, Fairmont State University, Fairmont, WV.* ^{*}*Department of Biochemistry and Molecular Medicine, WVU School of Medicine, Morgantown, WV.*

Dysregulated glucose metabolism is a hallmark of cancer, and emerging evidence suggests that localized glycolysis can support metastasis by generating a local supply of ATP to support cytoskeletal remodeling, membrane protrusion, and cell migration. However, the mechanisms of glycolytic enzyme recruitment to lamellipodia remain poorly understood. Our recent work suggests that filament formation by the liver isoform of the glycolytic “gate-keeper” enzyme phosphofructokinase-1 (PFKL) is required for its localization to lamellipodia and for directional sensing of chemotaxing breast cancer cells. We hypothesize that PFKL filaments interact with lamellipodial proteins to direct enzyme localization, thereby facilitating directional sensing and cell migration. To identify proteins which preferentially interact with filamentous PFKL, we performed APEX2 proximity labeling in cells expressing wild-type or filament-incompetent N702T APEX2-PFKL. Proteomic analysis identified proteins enriched in wild-type PFKL relative to N702T-PFKL (>2-fold, $p < 0.05$), including the PFK1 isoforms PFKP and PFKL, as well as focal adhesion-associated proteins DBNL, CAPNS1, and LRRFIP1. We prioritized DBNL because of its established role in cancer cell migration. We first examined whether DBNL and PFKL colocalize in lamellipodia, where immunofluorescent analysis of fixed cells demonstrated strong colocalization. We next tested whether PFKL forms a complex with DBNL by probing FLAG-PFKL immunoprecipitates with DBNL antibodies. DBNL did not co-immunoprecipitate with PFKL above control levels, suggesting that PFKL localization may instead depend on transient or indirect interactions. Future studies will use cross-linking immunoprecipitation to capture potential transient interactions between DBNL and PFKL. Additionally, siRNA-mediated DBNL depletion will determine a role in lamellipodia PFKL recruitment. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

4. Investigating the Effects of Synthetic Vape Coolants on Nicotine Reward-Related Behaviors.

Cameron Bailey, Brandon Henderson, Ph.D., Marshall University, Huntington, WV.

Flavor additives in electronic delivery systems (ENDS) have been shown to enhance nicotine reinforcement. Although some states and cities have pursued implementation of menthol bans, synthetic coolants such as WS-3 and WS-23 have emerged as substitutes. In this study, we investigated the impact of WS-3 and WS-23 on nicotine self-administration behavior and dopamine (DA) release in the nucleus accumbens (NAc) core. To model nicotine reinforcement, we used an E-Vape® self-administration (EVSA) assay and observed that male and female mice exposed to nicotine plus WS-3 and WS-23 exhibited enhanced self-administration behavior when compared to mice exposed to nicotine alone or PGVG (control treatment). We also observed that the enhancement in nicotine self-administration is similar to what is observed with menthol. To measure changes in dopamine release, we used fast-scan cyclic voltammetry (FSCV) and fiber photometry. Here, we observed that WS-3 and WS-23 increased the amount of dopamine released in the NAc under both tonic (5 Hz) and phasic (60 Hz) stimulation conditions when compared with nicotine-alone. Fiber photometry recordings showed that the WS-3 and WS-23 enhanced nicotine-stimulated dopamine release in the NAc core. Using electrophysiology and microscopy assays, we observed that synthetic coolants enhance the intrinsic excitability of ventral tegmental area (VTA) dopamine neurons and enhance nicotine-induced upregulation of nicotinic acetylcholine receptors (nAChRs). In both FSCV and photometry assays, the enhancement by synthetic coolants was similar to menthol-induced enhancements. These findings suggest that synthetic coolants enhance nicotine reinforcement, potentially by mechanisms identical to menthol. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

5. Investigating the Role of ERO1A in Murine Behavior Aaron J. Barbee*, Santhosh Shanthi Bhupathi*, Terri J. Poling†, James C. Walton†, Lori A. Hazlehurst*. *Cancer Institute, West Virginia University, Morgantown, WV †Department of Neuroscience, School of Medicine, West Virginia University, Morgantown WV

Endoplasmic Reticulum Oxidoreductase 1 Alpha (ERO1A) is an enzyme that involves protein folding, acting as a quality control supervisor and providing maintenance for balanced calcium homeostasis. The neurological function of ERO1A has been minimally studied, although ERO1A has been studied broadly in cancer and other diseases. This study focused on whether the removal of ERO1A affects neurological and behavioral function in mice. Due to ERO1A's role in maintaining calcium homeostasis, we hypothesized that the absence of ERO1A in mice would impair the neurological and behavioral performance in ERO1A knockout compared to wild-type mice. Sixteen ERO1A knockout mice and sixteen wild-type mice underwent a series of behavioral assessments. Anxiety was assessed using the Elevated Plus Maze, spatial recognition using the Y-Maze, exploratory behavior using the Open Field test, and motor coordination using the Rotarod test. Then neurological reflex was assessed using the Modified Neurological test, while memory was assessed using the Novel Object test, and finally pain using the Hot Plate test. Statistical differences were only found in the Rotarod test indicating that ERO1A plays a role in motor function in mice. The wild-type mice had better performance: longer latency to fall, faster RPM at fall. The absence of statistical differences in the remaining tests indicates that ERO1A does not play a significant role in baseline neurological functions or that other mechanisms are able to compensate for neurological performance. Future studies could explore other tests or increase the age of the mice to clarify the role of ERO1A in neurological function. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

6. Evaluation of the Antimicrobial Activity of Typhinamycin against Pathogenic Bacteria. *Amelia F. Bell**, *Joseph Horzempa**. **Department of Biological Sciences, West Liberty University, West Liberty, WV*

Antibiotic resistance continues to increase worldwide, highlighting the need to identify novel antimicrobial compounds. Typhinamycin, a purified fisetin-derived compound isolated from *Rhus typhina* (staghorn sumac), has previously exhibited antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. The objective of this study was to evaluate the antimicrobial activity of typhinamycin against a broader panel of clinically relevant bacterial pathogens. MRSA was included as a reference organism in each experiment and compared with two additional bacterial species. Standardized bacterial cultures were treated with typhinamycin, gentamicin (a control antibiotic), or were left untreated. Bacterial growth kinetics were monitored by hourly OD600 measurements over a 24-hour incubation using a plate reader. Typhinamycin inhibited the growth of several bacterial species, although the magnitude of inhibition differed among organisms, indicating species-dependent susceptibility. Additional studies, including determination of minimum inhibitory concentrations and characterization of its mechanism of action, are warranted to further evaluate its therapeutic potential against antibiotic-resistant infections. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

7. Spatial Distancing on Solid Media Determines Horizontal Gene Transfer of Plasmid DNA in *Pseudomonas aeruginosa*. Jaden B. Bender¹, Aimee M. Iradukunda², Noah J. Sauve², Newton Neog², Mackenzie A. Hall¹, Hayden T. Hess², Joseph Horzempa¹, and Yan-Yeung Luk², 1. Department of Biological Sciences, West Liberty University, West Liberty, West Virginia 2. Department of Chemistry, Syracuse University, NY.

Horizontal gene transfer (HGT) is the process of transferring genetic material between organisms through a process distinct from inheritance. This process allows bacteria to acquire new genetic material independently of reproduction. In this study, we sought to determine factors that influenced the rate of HGT in *Pseudomonas aeruginosa* bacteria. To do so, *P. aeruginosa* strains harboring either pMMB66EH (encoding carbenicillin [Cb] resistance) or pUCP26 (encoding tetracycline [Tc] resistance) were co-cultured at different densities and then plated on media containing both Cb and Tc. Bacteria co-cultured at high densities produced colonies with resistance to both antibiotics suggesting that the HGT outpaced antibiotic killing. To confirm that these bacteria possessed both plasmids, an isolate that exhibited resistance to both Cb and Tc was cultured and its plasmid DNA was purified using the Monarch Spin Plasmid Miniprep Kit from New England Biolabs. This DNA was digested with EcoRI & NheI and the restriction fragments were analyzed by agarose gel electrophoresis. This revealed DNA corresponding to the restriction fragments for the two expected plasmids (pMMB66EH at 8,807 base pairs and two bands for pUCP26 at 2,869 and 2,108 base pairs.) The pMMB66EH plasmid is a low copy plasmid and therefore exhibited a fainter band than either of the pUCP26 high copy plasmid bands. This analysis confirmed that HGT occurred here and provides insight into the rate of this phenomenon at the given density of bacteria. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

8. Synthesis, Structural Characterization, and In Vitro Antiproliferative Activity of Indole-Coumarin Hybrids against Breast Cancer Cell Lines. Rylyn A. Bielinski¹, Maryangeli Calderón-Sánchez², Zulma Ramos², and Eliud Hernández-O'Farrill². ¹West Liberty University, West Liberty, WV. ²University of Puerto Rico, Medical Sciences Campus, School of Pharmacy, Department of Pharmaceutical Sciences

Indole scaffolds exhibit anti-inflammatory and anticancer activities due to their heterocyclic structure, making them attractive candidates for developing novel anticancer agents. Additionally, coumarins exhibit favorable pharmacological properties, including low toxicity, high bioavailability and solubility, and diverse biological activities, such as anti-inflammatory and antiproliferative effects. We hypothesize that hybrid indole–coumarin derivatives will show potent anticancer activity against breast cancer cells. The study aims to synthesize an indole–coumarin derivative and evaluate its cytotoxic activity. The compound will be synthesized via carbon–carbon bond formation through a Knoevenagel condensation between indole-5-carboxaldehyde and 3,4,5-trimethoxyphenylacetonitrile, followed by N-alkylation of the indole with 3-(bromoacetyl)coumarin. The synthesized compound will be characterized by ¹H and ¹³C NMR and by GC–MS. We will evaluate the compound's cytotoxicity against the MDA-MB-231 and MDA-MB-435 breast cancer cell lines using the Sulforhodamine B assay. The expected result is that the indole–coumarin derivative will show significant antiproliferative activity in both cell lines. These findings will support the potential of indole–coumarin hybrids as pharmacophores for developing new anticancer agents. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

9. Targeted Mutation of FTL_1711 in *Francisella tularensis* Live Vaccine Strain. Audrey Blust* and Joseph Horzempa*. *Department of Biological Sciences, West Liberty University, West Liberty, WV.

Francisella tularensis is a pathogenic Gram-negative bacterium and the causative agent of tularemia. Naturally occurring subspecies of this facultative intracellular pathogen are known to cause human infections via blood-sucking arthropod vectors or contact with contaminated animal hosts. Considering the low infectious dose of this bacterium and its capacity to cause fatal pneumonic infection, the CDC classifies *F. tularensis* as a Tier 1 select agent due to the potential for misuse as a biological weapon. Previous work in our laboratory demonstrated that FTL_1199, a Fur-family transcriptional regulator in the *F. tularensis* Live Vaccine Strain (LVS), is required for erythrocyte invasion. Transcriptomic analysis identified FTL_1711 as downregulated in the Δ FTL_1199 mutant strain, suggesting that FTL_1711 may contribute to this phenotype. This current study aimed to generate targeted FTL_1711 disruption and deletion mutant strains for subsequent functional characterization. Mutagenic constructs were synthesized and subsequently mobilized into LVS by conjugation. For the disruption mutant, isolates were screened for chromosomal integration of the mutagenic plasmid. PCR confirmed that hygromycin-resistant disruption strains were bona fide mutants. For deletion of FTL_1711, two homologous recombination events were required for the elimination of this gene. Confirmation of the elimination of FTL_1711 was confirmed by PCR using primers flanking this gene. Studies using these mutant strains to determine the role of FTL_1711 in erythrocyte invasion are ongoing. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

10. Genetic Polymorphism in Lipid Droplet Protein CIDEA and Obesity in Mice *Jayden Cadle, Ana Chaguri, Jung Han Kim Department of Biomedical Sciences, School of Medicine, Marshall University, Huntington, WV.*

We proposed the cell death inducing DFFA like effector c (*Cidec*) gene, a lipid droplet protein, as a candidate for an obesity QTL identified in obese, diabetic TALLYHO/Jng (TH) mice. There was a missense variant in *Cidec* (R46S). We generated *Cidec*^{R46S} knock-in (KI) mice where the S46 is exchanged for the R46 at *Cidec* on C57BL/6J (B6). This study was to characterize *Cidec*^{R46S} KI mice for obesity susceptibility. Male and female B6 and KI mice were weaned onto chow or high fat diets (HF). At 14 weeks of age (wk), body composition, energy expenditure (EE), and food intake (FI) were measured. At 16wk, mice were euthanized and gonadal fat protein expression levels of *Cidec* and adipose triglyceride lipase (*Atgl*) were measured by western blot analysis. Data were analyzed by 2 way-ANOVA followed by multiple comparisons post-tests with Tukey's correction. A p-value of 0.05 was required for significance. For males, KI mice were slightly heavier than B6 on HF, with increased EE. For females, KI mice had slightly larger fat mass than B6 on HF. CIDEA expression was higher in mice fed chow vs HF. In males, KI mice exhibited higher CIDEA levels than B6 on chow. For ATGL levels, there was also a significant diet effect (lower on HF vs chow), regardless genotype, for males, while KI females exhibited higher on HF vs chow. The missense variant in mouse *Cidec* may confer diet-induced obesity minimally, with limited association with protein levels of *Cidec* and *Atgl*. **(Supported by AHA UGR: 25IAUST1377724, AHA Marshall Summer Undergraduate Research Experience; JCESOM Matching funds and NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

11. Identifying Genetic Factors for Obesity and Hypercholesterolemia on Chromosome 1 of TALLYHO/JngJ Mice. Ana Chaguri, Jayden Cadle, Jung Han Kim. Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University

Obesity and hypercholesterolemia are polygenic disorders. Previously, we located a QTL, conferring hypercholesterolemia and diet-induced obesity, to the distal region of chromosome 1 (~16.2Mb in size) in obese, diabetic TALLYHO/Jng (TH) mice. Subsequently, we fixed two overlapping congenic lines, A and B, carrying TH segment of the QTL on C57BL/6J (B6) background. The present study was to characterize these two lines of mice and evaluate a candidate gene. Male and female congenic lines and B6 mice were weaned onto chow or high fat diets (HF). At 14 weeks of age (wk), body composition was measured. At 16wk, mice were euthanized and the blood and gonadal fat samples collected. Plasma total cholesterol levels were determined using colorimetric assays and adipose tissue protein levels of the *Aim2* gene by western blot analysis. Data were analyzed by 2 way-ANOVA followed by multiple comparison post-tests with Tukey's correction. A p-value of 0.05 was required for significance. On chow, cholesterol levels were higher in lines A and B than in B6. On HF, line A had significantly larger fat mass and cholesterol levels than B6, while line B showed higher cholesterol levels than B6, with comparable fat mass. These results suggest that the QTL candidate region is likely located within the overlapping segment between lines A and B for hypercholesterolemia, while within the unique segment retained in line A for obesity. Protein levels of *Aim2*, a candidate for diet-induced obesity, were increased in line A, but not in line B, compared to B6. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

12. The Na⁺/K⁺-ATPase α 1 Signaling Pathway in Adverse Cardiac Remodeling Vinu

Chandrasekar¹, Sidney Strause², Karleigh Burdette², Marco T. Pessoa², Liquan Cai², and Sandrine V. Pierre² ¹Marshall University, Huntington, WV ²Joan C. Edwards School of Medicine, Department of Biomedical Sciences, Huntington, WV

Cardiovascular disease is the leading cause of death worldwide, with heart failure (HF) accounting for a significant portion of mortality. During HF progression, cardiac Na/K-ATPase (NKA) α 1 isoform expression declines. Although NKA α 1 functions as both an ion pump and a Src-dependent signaling receptor, the respective contribution of signaling functions to the progression of adverse cardiac remodeling in HF remains unclear. To isolate NKA α 1 signaling function from ion transport, we used a Myh6-Cre/Lox approach to generate cardiomyocyte-specific Atp1a1 knockout mice. We then rescued cardiac NKA ion-pumping activity by crossing with mice expressing a transgene NKA α 1 or NKA α 2 under a Myh-6 promoter. Because NKA α 2 maintains ion transport but lacks Src-dependent signaling, this model distinguishes NKA signaling-dependent effects from ion pumping activity. Western blotting of cardiac tissue preparations confirmed the expression of the respective rescued NKA polypeptides (n=3). Mice were infused with angiotensin II (1.5 mg/kg/day) for 14 days to induce cardiac remodeling. Both rescue groups developed comparable cardiac hypertrophy (~20% increase in heart weight/body weight; NKA α 1: 4.728 vs. 5.773 mg/g, NKA α 2: 4.813 vs. 5.837 mg/g; n=3–5, p<0.05). However, RT-qPCR showed that NKA α 1-rescued mice exhibited greater type 1 collagen (COL1A1) expression than NKA α 2-rescued mice (n=3–4, p<0.05). These findings suggest that Src-dependent NKA α 1 signaling may promote collagen production during hypertrophic stress and contribute to adverse cardiac remodeling. **(Supported by American Physiological Society Summer Undergraduate Research Fellowship (PI: Chandrasekar), American Heart Association 26PRE1544880 (PI: Strause) and 25TPA1477521 (PI: Pierre), and NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.)**

13. The role of chemokines in habenular axon outgrowth in zebrafish. *Dakota Charles*, Gabrielle Justice*, Anthony Poe*, Din Rayche*, Wilthin Alcime*, Sara Roberson*. *Department of Applied Science, Bluefield State University, Bluefield WV.*

The habenula (HB) is a bilateral structure of the dorsal forebrain, conserved among all vertebrates. It is responsible for a variety of behaviors such as fear/anxiety, reward vs punishment, and sleep. Abnormal habenular function has been linked to psychiatric diseases such as major depressive disorder and bipolar disorder. The axons of the HB all project posteriorly from the forebrain to the interpeduncular nucleus (IPN) in the midbrain. Previous work has shown that chemokine signaling is required for correct axon projection in the posterior direction, with disruptions in chemokine signaling leading to aberrant anterior axon projections. In zebrafish, the chemokine receptor Cxcr4b is produced in the HB, and there are two chemokine ligands, Cxcl12a and Cxcl12b, expressed in the region around the developing HB. While both ligands have been implicated in assisting the axons to project in the correct anterior-posterior direction, the exact roles of these ligands are unknown. This work aims to explore the individual roles of Cxcl12a and Cxcl12b in habenular axon outgrowth. Preliminary data show that overexpression of *cxcl12a* does not result in the anterior axon projection phenotype seen with the loss of Cxcl12a. Conversely, very early data suggest that loss of Cxcl12b does not result in the abnormal phenotype, while overexpression of *cxcl12b* does. This suggests a more complicated relationship between the two ligands and axon outgrowth. To this end, we are mapping the respective expression domains of the two ligands in relation to the growing HB axons. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

14. Anatomical Mapping and Functional Imaging of Stress-Responsive Modulation of Sensory Processing. *Manuella Cogliatti†, Lindsey Morais*, Ph.D. Ramiro Comas Mutis*, Ph.D. Morgan Bridi*, †-Department of Biology and Environmental Science, Davis and Elkins College, Elkins, WV
Department of Neuroscience, West Virginia University, Morgantown, WV.

Stress is defined as any challenge that disrupts physiological homeostasis and is a major contributing factor in numerous neuropsychiatric disorders, including post-traumatic stress disorder (PTSD) and anxiety disorders. Corticotropin-releasing factor (CRF) is a neuropeptide best known for initiating the hypothalamic-pituitary-adrenal (HPA) axis through neurons of the paraventricular nucleus (PVN), ultimately promoting glucocorticoid release during stress. Although CRF is also present within the sensory cortical regions, including the primary auditory cortex (ACx) and the Primary Visual Cortex (V1), the origin and functional significance of these CRF inputs remain unclear. We hypothesize that PVN-CRF neurons project to sensory cortex and contribute to stress-induced modulation of sensory processing. To test this hypothesis, anterograde and retrograde viral tracing will be used to identify CRF+ projection from the PVN to ACx, V1, and other cortical areas. Following anatomical characterization, widefield calcium imaging will be used to examine how CRF/CRFR1 signaling influences sensory cortical activity, measured before and after local infusion of a CRFR1 agonist. These studies will improve our understanding of how stress-related circuits regulate sensory processing and may provide insight into mechanisms underlying altered sensory function in stress-associated disorders. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA network for Biomedical research Excellence).***

15. Antimicrobial Activity of *Verbascum thapsus* Essential Oil Nanoemulsion Against Drug-Resistant Bacteria Jonathan Cooper, Mukesh Sivakumaran, and Shreyas Varathan West Virginia School of Osteopathic Medicine, Lewisburg, WV.

An increase in antibiotic-resistant bacterial strains, along with the decreased pace of new antibiotic development, has caused a search for alternative therapeutics, including essential oils. The antimicrobial activity of *Verbascum thapsus* Essential Oil Nanoemulsion (VEO NE) against various drug-resistant bacterial species remains incompletely understood. There is existing data pointing to the effectiveness of the antimicrobial properties of other essential oil nanoemulsions. Tests of the effectiveness of VEO NE against Gram-positive bacteria, including *Staphylococcus epidermidis* and *Bacillus subtilis*, and Gram-negative species, including *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were performed. Three assay methods were used to collect data: disk diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC). Disk diffusion assay was used to test for the effectiveness of different concentrations of VEO NE at 2.5%, 5%, 7.5%, and 10% against these bacteria by measuring the zone of inhibition on Mueller-Hinton agar plates. For the positive controls, Ciprofloxacin, Penicillin, and Gentamicin antibiotics were used against these bacteria. The negative control consisted of nanoemulsion samples containing only Tween 80 and water, without any essential oil. There was a general trend with the higher concentration of VEO NE showing a larger zone of inhibition (ZOI). An exception to this was *Pseudomonas aeruginosa*, which showed no ZOI for any concentrations. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

16. Bidirectional, Sex-Dependent Effects of Rosiglitazone on Astrocyte–Synapse Remodeling and Behavior Following Adolescent Binge Ethanol Exposure *Kadence Crank¹, Darshan Sangani¹, Tristan Muck¹, Olivia Coulter¹, Mika Jones^{1,2}, Hannah Sexton^{1,2}, Mary-Louise Risher^{1,2}* ¹*Department of Biomedical Research, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25701, USA* ²*Neurobiology Research Laboratory, Hershel “Woody” Williams Veterans Affairs Medical Center, Huntington, WV 25704, USA*

Adolescent binge drinking can result in long-term behavioral dysregulation and increases the risk of developing an alcohol-use disorder. Using the adolescent intermittent ethanol (AIE) rat model, we previously demonstrated maladaptive changes in the contextual fear conditioning task that were associated with dorsal hippocampal (dHipp) astrocyte reactivity, aberrant astrocyte signaling, tripartite structural remodeling, and astrocyte and neuronal cellular stress. Additionally, we observed that Rosiglitazone (ROSI), a drug developed for type 2 diabetes, may be an effective therapeutic for reducing neuron and astrocyte-associated oxidative stress. We hypothesize that ROSI administration is sufficient to rescue AIE-induced oxidative stress, tripartite structural remodeling, and cognitive deficits in adulthood. On PND 24, male and female Sprague-Dawley rats underwent AIE or H₂O. Animals then received ROSI or Vehicle for 20 days. In adulthood, animals underwent behavioral testing or were euthanized for tissue collection for immunohistochemistry (IHC), stimulated depletion emission microscopy (STED), or confocal microscopy to evaluate tripartite proximity and markers of cellular stress. Results demonstrate that AIE heightened fear responding in males and accelerated fear extinction in females, both effects were rescued by ROSI administration. We observed a sex-dependent increase in peripheral astrocyte process (PAP) proximity to inhibitory synapses and loss of proximity at excitatory synapses in the dHipp that was rescued by ROSI administration. Results evaluating cellular stress in dHipp will also be presented. Future studies will investigate the region-specific effects of AIE and ROSI in other brain regions associated with fear regulation. These data suggest that Glitazone-based compounds may have promising therapeutic potential. **(Funding: Department of Veterans Affairs (BLR&D) IBX005403 and the National Science Foundation 2242771. (Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

17. Kaempferol reduces cytotoxicity of hydrogen peroxide and cisplatin in renal proximal tubule cells *Kiersten Davis+, Christopher Bender*, Katie Brown*, Elizabeth McGuffey*, Monica Valentovic** *Department of Biomedical Sciences, Toxicology Research Cluster, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV;+Fairmont State University, Fairmont, WV

Renal function is impaired by drugs and environmental chemicals which increase the generation of reactive oxygen species (ROS). Recent studies have tested whether natural products could reduce renal damage induced by drugs or ROS components such as hydrogen peroxide (H₂O₂). Kaempferol (KAEMP) is an antioxidant with anti-inflammatory properties widely found in fruits, dark green vegetables, legumes, and other plants. Previous studies reported the beneficial effects of KAEMP in reducing cancer cell growth. This study tested whether KAEMP would reduce cisplatin or H₂O₂ cytotoxicity in HK-2, a human noncancerous renal epithelial cell line. HK-2 cells were pretreated for 1h with 0-20 uM KAEMP followed by a 24h exposure to 0-1000 uM H₂O₂ or 0-50 uM cisplatin. This model utilized cisplatin; a platinum-based chemotherapy drug used to treat various cancers, but identified to cause nephrotoxicity via damaging DNA, inhibiting cell division, increasing ROS and ultimately leading to irreversible injury and apoptosis. The HK-2 cells were first grown for 48h, exposed to KAEMP for 24h, and then exposed to cisplatin or H₂O₂ for 24h. MTT was increased ($p < 0.05$) after 24h by 10-20uM KAEMP relative to vehicle control suggesting improved mitochondrial health. KAEMP prevented the decline in MTT by 500uM H₂O₂ and 30 or 50 uM cisplatin. Western blot evaluated the effect of KAEMP on NADPH Oxidase 4 (NOX4) and autophagy markers LC3BII/I following exposure to H₂O₂. In conclusion, KAEMP was shown to promote mitochondrial health and has the capability to prevent loss of kidney function mediated by cisplatin. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence and NIH Grant U54GM104942-08).***

18. The *Pseudomonas aeruginosa* 1244 pilin glycan enhances twitching motility and renders bacteria more susceptible to human beta defensin 2. Shania Davis & Joseph Horzempa, Department of Biological Sciences, West Liberty University, West Liberty, WV USA.

Pseudomonas aeruginosa is an ESKAPE (highly drug resistant) pathogen that is commonly acquired during hospital stays. This bacterium produces type IV pili which are adhesins and motor appendages that mediate a surface motility referred to as “twitching.” These pili are polymers of protein subunits referred to as pilin. The pilin subunit of *P. aeruginosa* 1244 is glycosylated. Previous studies have shown that the pilin glycan affects the efficiency of twitching motility under certain conditions, fiber surface polarity, and overall bacterial surface polarity. We also confirmed and extended these previous findings by showing pilin glycosylation substantially enhances twitching motility on positively charged surfaces (poly L-lysine coated plastic). Because modulation of the bacterial surface charge has been shown to mediate defensin resistance in other organisms, we tested whether pilin glycosylation affected sensitivity to human beta defensin 2 (HBD-2). Interestingly, the isogenic mutant strain lacking the pilin glycan (1244G7) showed increased resistance to HBD-2 compared to wild type bacteria and those completely lacking a type IV pilus (1244.47). This increased resistance to HBD-2 could explain data from a recent study in which various *P. aeruginosa* clinical strains were isolated that had pilin glycosylation defects. We therefore sought to determine if pilin glycosylation modulates the interaction between pili and HBD-2. Here, an immunoprecipitation assay was carried out using HBD-2 as bait and either purified glycosylated pili or non-glycosylated pili as bait. These studies are ongoing and could provide insight into an important host-pathogen interaction. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

19. Investigating NDRG1 and Rb Interactions in Clear Cell Renal Cell Carcinoma. *Ashton Dean¹, Chelsea McCallister², Sam Roth², Travis Salisbury². ¹University of Charleston, Charleston, WV. ²Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, 1 John Marshall Drive, Huntington, WV*

Clear cell renal cell carcinoma (ccRCC) accounts for over 80% of all kidney cancer cases and is characterized by the dual-allele loss of the Von Hippel-Lindau (VHL) gene. We have shown that in 786-0 cells, VHL null cells, the protein N-Myc Downstream Regulated 1 (NDRG) stabilizes Retinoblastoma protein (Rb). One question is whether this linear relationship between NDRG1 and Rb is dependent on the absence of VHL. We hypothesized that in VHL positive HK2 cells, when VHL is reduced, NDRG1 and Rb protein levels will be increased. In order to study this relation, VHL was knocked down via transfection, using two different siRNA targeting VHL. After transfection, cells were extracted for western blot analysis to determine relative protein levels. It was observed that in response to a ~85% VHL decrease, NDRG1 remained at relatively similar levels, and Rb levels increased by ~70%. These data suggest that in acute response, Rb levels in HK2 change directly in response to VHL rather than by NDRG1 through the VHL-NDRG1-Rb pathway. We theorize that in order to see the effect of NDRG1 on Rb in normal renal cells, cells may need a larger abundance of NDRG1 or hypoxic conditions to induce a stress response. To examine if hypoxic conditions change this relationship in HK2 cells, we will run the same transfection in 5% oxygen conditions. Another future direction is to examine this relationship in patient-derived cell lines. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

20. Defining Adolescent Tripartite Synapse Maturation Using Integrated Spatial Transcriptomics and Stimulated Emission Depletion (STED) Microscopy *Claire Denning¹, Nisha Kumar¹, Mika Jones¹, Aaliyah Walls¹, Grace Angelona¹, Tristan Muck¹, Faith Malone¹, Olivia Coulter¹, Hannah Sexton^{1,2}, Krista Denning³, Hadi Ul Bashir⁴, Sadia Akter¹, Mary-Louise Risher^{1,2}. ¹Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25701, USA; ²Neurobiology Research Laboratory, Hershel 'Woody' Williams Veterans Affairs Medical Center, Huntington, WV 25704, USA; ³Department of Pathology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25701, USA; ⁴Department of Biological Sciences, Marshall University, Huntington, WV 25755, USA*

Adolescence is a critical period of brain development characterized by extensive synaptic remodeling that supports cognitive function, decision-making, and flexibility. Many synapses are ensheathed by peripheral astrocyte processes (PAPs) to form the tripartite synapse where astrocyte–synapse interactions regulate neuronal communication. Our laboratory has previously demonstrated that adolescent intermittent ethanol exposure (AIE) induces a subregion-dependent loss of PAP-synaptic proximity within the orbitofrontal cortex (OFC). We propose developmental differences in tripartite synapse maturation may contribute to alcohol-induced dysfunction; however, the normal developmental trajectory of tripartite synapse maturation remains understudied. To investigate structural and molecular changes in the developing tripartite synapse during adolescence, male and female C57BL/6 mice were euthanized at postnatal day (PND) 31 (early adolescence), PND 46 (late adolescence), or PND 72 (adulthood). Brain tissue was collected for stimulated emission depletion (STED) microscopy and Visium HD spatial transcriptomics (10x Genomics). STED imaging quantified developmental changes in PAP-synaptic proximity, while spatial transcriptomics characterized age-dependent gene expression patterns associated with tripartite synapse maturation. By integrating nanoscale structural imaging with spatially resolved transcriptomics, this study will provide needed context for future studies on the relationship between AIE-induced loss of PAP-synaptic proximity and the loss of synapse stabilizing proteins. These findings may provide insight into why the adolescent brain is particularly susceptible to the long-term effects of alcohol exposure. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence) Funding: National Science Foundation, RII Track 1: WV-NFNT, Grant# NSF-2242771 to Janet Rorrer (Principal Investigator) and ML Risher (Research Lead); Department of Veterans Affairs (BLR&D) BX005403.**

21. Active Hexose Correlated Compound Enhances Macrophage Stimulation in Stressed Mice during *Chlamydia muridarum* Genital Infection. *Isabella Dibbisio, Gebreegzabher Belay, Tesfaye Belay. Bluefield State University, Bluefield, WV.*

Chlamydia genital infection is the most common genital infection worldwide. The prevalence of this disease is high in low-socioeconomic status populations. Active Hexose Correlated Compound (AHCC) is a dietary supplement to boost the immune system. While macrophages play a crucial role in antigen presentation, the effects of AHCC feeding of stressed mice are poorly documented. This study explores the gene expression and secretion of proinflammatory cytokines and major surface markers in macrophages of stressed mice. We hypothesize that AHCC feeding to stressed mice enhances the gene expression and secretion of proinflammatory cytokines and expression of surface markers in macrophages. AHCC-fed BALB/c mice were stressed for 5 minutes daily for 21 days & infected intravaginally with *C. muridarum*. After 7 days of infection, bone marrow-derived macrophages and mature intestinal macrophages were purified, cultured, and RNA was isolated for gene expression. Culture supernatants were collected to determine cytokine production by ELISA. Gene expression of proinflammatory cytokines IL-1 β , IL-6, and TNF- α , and major surface marker expression on macrophages, and signature cytokine production profiles were determined by qPCR and flow cytometry. ELISA results show that AHCC increases cytokine production by macrophages compared with stressed PBS-fed mice. Higher expression of macrophage surface markers was obtained in AHCC-fed mice compared to PBS-fed stressed mice. Overall, AHCC feeding increases the production of protective and suppressive cytokines by macrophages. The obtained preliminary data suggest that AHCC has immunomodulatory effects via immune cell activation, including macrophages, which could be helpful for further studies in stressed physiological animal and human subjects. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence).**

22. Optimization of Acetalated Dextran Nanoparticles for Targeted Drug Delivery. *Kaitlynn Drainer, Rukiye Nurseker, Sharan Bobbala. *Department of Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Morgantown, WV*

Nanoparticles allow the encapsulation of chemotherapy drugs and enhance their systemic circulation times and accumulation in the tumor microenvironment. These drugs demand targeted delivery to cancer cells to achieve the highest therapeutic efficacy while minimizing undesired side effects. We have recently developed acetalated dextran (Ac-Dex) nanoparticles that can passively accumulate in the bone marrow tumor microenvironment in B-cell acute lymphoblastic leukemia (B-ALL). The targeting ability of Ac-Dex nanoparticles to B-ALL cells may be enhanced by decorating ligands, such as monoclonal antibodies (mAbs), on the nanoparticle surface. However, surface conjugation of an antibody to a nanoparticle surface requires high-precision design and control of physicochemical characteristics. Here we have utilized PEGylated lipids (DSPE-PEG and DMG-PEG) and optimized formulation conditions to achieve surface conjugation of mAbs on Ac-Dex nanoparticles. Ac-Dex polymer was synthesized from Dextran, and nanoparticles were formulated using the flash nanoprecipitation technique. We activated the thiols of anti-human CD19 mAb to allow conjugation to maleimide-functionalized PEGylated lipids on the nanoparticle's surface. CD19 mAb conjugated DMG-PEG and DSPE-PEG Ac-Dex nanoparticles demonstrated nanoparticle sizes of 202 and 254 nm respectively, with low polydispersity index (<0.3). mAb conjugation efficiency was found to be 28-30%. In addition, DMG-PEG and DSPE-PEG Ac-Dex nanoparticles allowed successful encapsulation of physicochemically diverse payloads. Overall, these findings showcase the suitability of mAb conjugation to Ac-Dex nanoparticles through clinically relevant PEGylated lipids and further demonstrate the potential of this delivery platform for targeted cancer treatments. **(Supported by NIH Grant P20GM103434 to the West Virginia Idea Network for Biomedical Research Excellence)**

23. Sex-Dependent Modulation of Induced-Thyrotoxicosis in Developing Chicken Embryos

Logan Eddy, Nicole Garrison, and Holly Vopal Department of Biological Sciences, West Liberty University, West Liberty, WV

Hyperthyroidism is when the body produces excess thyroid hormones that can facilitate thyrotoxicosis. Thyrotoxicosis in pregnant women increases the risk of craniosynostosis (CS) in the fetus. CS is a condition where sutures of the skull fuse prematurely during development *in utero*. Our lab utilizes an avian model of induced thyrotoxicosis and have observed morphological and molecular alterations with thyroxine exposure. However, the impact of the embryo's sex has not yet been considered. In humans, CS occurs more often in males than females (3:1). Additionally, studies indicate that maternal thyrotoxicosis impacts male fetuses more significantly regarding growth and development. Male chicken embryos, like humans, show higher metabolic activity and exhibit accelerated growth compared to females. Increased metabolism of thyroxine by the liver may also therefore be altered with thyrotoxic conditions. We hypothesize to observe sex-dependent modulation with our thyroxine treatment. To determine sex, we collected toe clips from the vehicle control and thyroxine treated embryos and performed PCR to identify the W chromosome (females) by targeting both the SWIM and CHD genes using custom primers. Since the SWIM gene is located on the W chromosome, electrophoresis results in a single band for females only, while CHD served as our positive control found on both Z and W chromosomes. Comparison were made between both sex and treatment for body mass and length measurements as well as for liver deiodinase (DIO) expression. While significant findings were observed between treatment and controls, we did not observe differences between determined sexes. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

24. Expression profiling of soleus muscle in TALLYHO and C57BL/6 mice. *Sophia Ellingson, West Virginia State University; Dr. James Denvir, Marshall University; Dr. Jung Han Kim, Marshall University; Kristiana Skiffington, Marshall University*

The TALLYHO (TH) is a strain of polygenic mouse that is insulin resistant at birth. The C57BL/6 (B6) is a strain of isogenic mouse that is only insulin resistant on a high fat diet and is used often as a comparison in mouse studies. This study was conducted on the male mice of the TH and B6 strains that were aged 20 weeks on a standard chow diet and in the tissue of the soleus muscle. The soleus muscle, found in the thigh area, is a highly insulin-sensitive tissue because it is involved in glucose uptake. We performed bioinformatic analysis of RNA-Seq data from the soleus muscle of these strains. This analysis revealed 4,625 differentially expressed genes between TH and B6. Gene Set Enrichment Analysis identified 23 activated and 46 inhibited biological processes. The *Antigen Processing and Presentation* process was representative of many of the inhibited pathways. A relationship between antigen processing and insulin resistance is well-supported in the literature, making for a mechanistic hypothesis as to why TH mice are insulin resistant. This specific pathway is the process in which an antigen presenting cell expresses antigen (peptide or lipid) on its cell surface in association with a Major Histocompatibility Complex (MHC) protein complex. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

25. Biotherapeutic Potential of Bacterial Species Living on Plants in Southern West Virginia.

Sydney Feazell, OMS-II, Matthew Williams PhD West Virginia School of Osteopathic Medicine

The rising demand in natural probiotics has increased interest in identifying microorganisms residing on plants traditionally associated with having medicinal properties. In this study, bacterial isolates were extracted from wild ramps (*Allium tricoccum*) growing in southern West Virginia. This plant was selected due to its widely known health benefits to humans, including gut health, lowering cholesterol and blood pressure, and cardiovascular function. Bacterial isolates were tested against five common human pathogens: *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Serratia marcescens*. Both isolates showed strong antagonistic activity against all five pathogens on both antagonism and headstart assays, allowing us to examine these samples further into their potential for biotherapeutic use. Supernatant assays were also performed and showed negative results on both isolates, suggesting that no nonspecific antimicrobial was secreted. DNA extraction was performed on these samples and using 16S sequencing analysis with comparison to the NCBI database it was determined that these isolates were most similar to *Paenibacillus lautus* for the first sample and *Bacillus spp.* for the second sample. These findings suggest that medicinal plants surrounding West Virginia may harbor bacterial species with potential antimicrobial activity. However, further research into these novel strains are needed to identify how they are able to modulate the human microbiome and their potential use as biotherapeutic agents. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

26. Nephrotoxicity and the Mechanism of 2,6-Dichlorohydroquinone in Isolated Rat Kidney Cells. *Maeghan B. Fisher, Cade Cole, Mika McCormick, Dianne K. Anestis, Gary O. Rankin, Department of Biomedical Sciences, Marshall University, Huntington, WV, Department of Science and Technology, Fairmont State University, Fairmont, WV.*

Industrial compounds, drugs, and pesticides have been found to use chlorinated benzenes as intermediates during synthesis. Chlorinated benzenes have been found to be nephrotoxic in several species. Studies show that 1,3-dichlorobenzene is toxic via metabolites, especially phenolic metabolites. Those phenols can be metabolized to become hydroquinones, including 2,6-dichlorohydroquinone (2,6-DCHQ). 2,6-DCHQ redox cycles producing 2,6-dichlorobenzoquinone (2,6-DCBQ) and oxygen free radicals. 2,6-DCBQ has 2 carbonyl groups and the potential to bind to enzymes or proteins. The purpose of this research was to examine the nephrotoxic potential of 2,6-DCHQ and use antioxidants and an iron chelator to determine the role of free radicals in 2,6-DCHQ nephrotoxicity. Isolated kidney cells were obtained from male Fisher 344 rats. Cells (~4 million/mL, 3 mL) were exposed to DMSO (control) or 2,6-DCHQ (0.1 mM-1.0 mM) for 30 and 60 minutes. In some experiments, five-minute pretreatment with deferoxamine, ascorbate or α -tocopherol, or a 30-minute pretreatment with glutathione was performed. Toxicity was determined by measuring lactate dehydrogenase (LDH) release. Results showed that at 0.25 mM and above, 2,6-DCHQ is cytotoxic, with 1.0 mM increasing in toxicity between 30 and 60 minutes. Deferoxamine, glutathione, ascorbate, and α -tocopherol significantly reduced 2,6-DCHQ toxicity. In conclusion, 2,6-DCHQ induced toxicity at 0.25 mM or higher. Ascorbate, glutathione, and α -tocopherol, reduced toxicity of 2,6-DCHQ indicating a role for free radical in toxicity development. Deferoxamine provided full protection which shows that the toxicity is due to hydroxyl radicals. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

27. Impact of Early Life Stress and Gestational Stress on Relevant Behaviors in Pregnant Mice: Implications for Peripartum Mental Health Disorders *Makel E. Galloway, Marissa M. Nicodemus, Kathleen E. Morrison* *Department of Psychology, West Virginia University*

Women are at a greater risk of anxiety and mood disorders compared to men, although this risk is multifactorial and not well understood. Both becoming pregnant in adulthood and exposure to stressors during the lifespan, including during early life or during pregnancy, are known risk factors for perinatal anxiety and mood disorders. Using our translationally relevant mouse model, we previously showed that chronic variable stress (CVS) during puberty led to a disruption of the hormonal stress response in adulthood, but only when females were pregnant. Here, we extended that work to examine the additional impact of gestational stress and to examine mood-relevant behaviors. We hypothesized that exposure to both pubertal CVS and gestational stress in adulthood would lead to altered anxiety- and depressive-like behaviors. Female mice were exposed to CVS during postnatal days 21-34 or not (control). During pregnancy in adulthood, they were exposed to either a limited nesting material (LNM) stressor or received standard bedding. Mice were evaluated in the splash, social interaction, sucrose preference, and open field tests in late pregnancy. We found a significant main effect of pubertal CVS on sucrose preference, with CVS-exposed pregnant mice displaying a greater sucrose preference regardless of bedding conditions. We found no effect of CVS or LNM on other measured behavior outcomes. Overall, this work extends our understanding of the risk factors for disrupted mood and anxiety throughout the female lifespan. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence and NIH Grant HD091376 to KEM)**

28. Assessment of Movement and Motivation Deficits in C57BL/6J Mice Before and After Photothrombotic Stroke: Towards an Ensemble-Based Strategy for Improving Post-Stroke Recovery Maggie Gunter^{1,3}, Ekaterina Weil, PhD¹; Vincent Setola, PhD^{1,2} ¹Department of Neuroscience, ²Department of Behavioral Medicine and Psychiatry, West Virginia University School of Medicine Health Sciences Center, Morgantown, WV; ³West Liberty University Center for Biomedical Research, Wheeling, WV

Motivational deficits are among the most common yet least understood consequences of stroke, often reducing quality of life and hindering rehabilitation. Current treatments targeting neurotransmitter systems are frequently only partially effective. It may be that targeting neuronal ensembles involved in motivation would be more therapeutic. Here we report a paradigm for a future ensemble-based approach to investigating post-stroke motivation. Male and female wild-type C57BL/6J mice were trained to perform sucrose self-administration (SSA) in operant chambers equipped with levers, dippers, and cue lights. Mice that successfully acquired SSA (“acquirers”) underwent progressive-ratio (PR) breakpoint testing to quantify motivation; they were also assessed in open-field locomotor chambers to characterize and quantify baseline locomotion. Subsequently, acquirers received a mild-moderate midline photothrombotic stroke (PTS) targeting the cingulate/M2 motor (CgM2) cortex. Following recovery, mice repeated SSA breakpoint and open-field locomotor testing to assess stroke-induced changes in motivation and locomotor activity, respectively. Laser speckle imaging was used to confirm the presence of infarcts in each of the six acquirers post-stroke. Whereas PTS did not affect locomotor activity relative to pre-stroke values, post-stroke breakpoint testing showed post-stroke decreases in breakpoint for two of the mice. Two other mice had identical breakpoints pre- and post-stroke, and the two remaining mice had slight increases in SSA breakpoint. These data suggest that only some mice receiving CgM2 PTS develop motivational deficits, suggesting additional optimization of PTS parameters will be needed prior to launching studies using TRAP2/DIO::DREADDs to re-activate SSA-relevant ensembles to determine the effects of ensemble re-activation/inhibition on post-stroke motor deficits. **Supported by NIH Grants P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence) and P30 GM159569 (WV Stroke CoBRE grant)**

29. Development of a Subarachnoid Hemorrhage Mouse Model to Study Artificial Light at Night on Patient Outcomes. *Madalyn Harless^{1,2}, Tara K.S. Craft¹, Terri J. Poling¹, James C. Walton¹, Kate Karelina¹, A. Courtney DeVries^{1,3}, Randy J. Nelson¹. ¹School of Public Health, ²Department of Neuroscience, Rockefeller Neuroscience Institute, ³Department of Medicine, West Virginia University, PO Box 9303, Morgantown WV, 26506, USA*

Subarachnoid hemorrhage (SAH) is a form of stroke caused by bleeding into the region between the arachnoid membrane and pia mater surrounding the brain. It affects 10 to 14 per 100,000 individuals annually in the United States. SAH carries a mortality rate of 25–40%, and more than 50% of survivors experience lasting cognitive and neurological disability. SAH is a critical public health issue, encompassing high treatment costs, prolonged rehabilitation, and a significant prevalence of modifiable risk factors such as smoking and hypertension. The lack of progress in clinical trials creates the need to develop animal models to better understand the underlying mechanisms driving poor outcomes. We have recently reported that disruption of circadian rhythms by exposure to dim artificial light at night (ALAN) worsens outcomes in other forms of ischemia (ischemic stroke and cardiac arrest); thus, we hypothesized dim ALAN exposure would affect SAH outcomes. Male and female CFW mice were housed under standard light-dark cycles or light-dim ALAN (5 lux) conditions for four or more weeks prior to SAH surgery, followed by seven days of physiological and behavioral assessments, including body mass measurements, Clark deficit scoring, and open field testing. ALAN had no significant effect on body mass, survival, or Clark deficit scores. ALAN exposure produced an anxiety-like phenotype in male, but not female, mice post-SAH, reflected by reduced central tendency in open field testing. These results contribute to the understanding of how light at night exposure and sex may interact to shape recovery outcomes of SAH. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

30. Investigating Appalachian Plants as a Source of Next-Generation Biotherapeutics *Kyle Hawthorne, Matthew Williams*

More than 500 bacterial species are recognized as human pathogens capable of causing infectious disease. These pathogens establish infection through mechanisms including competition with the native microbiota for nutrients, invasion of host tissues, and, in some cases, production of toxins that disrupt normal physiological processes. As antimicrobial resistance continues to increase, the identification of naturally occurring bacteria with antagonistic activity against clinically relevant pathogens represents a promising avenue for the development of novel biotherapeutics. In this study, bacterial isolates were obtained from the surfaces of Adam's Needle (*Yucca filamentosa*) and Northern Red Oak (*Quercus rubra*), two plant species historically associated with the treatment of sores in traditional Appalachian medicine. Plant samples were collected from multiple counties throughout West Virginia. Antagonism assays were performed to evaluate the inhibitory activity of five Appalachian bacterial isolates (KG14, KG15, KG16, MPf, and MPg) against five clinically relevant bacterial pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans*, *Serratia marcescens*, and *Klebsiella pneumoniae*. The objective was to determine whether naturally occurring plant-associated bacteria possess antimicrobial activity against common human pathogens. Our results demonstrate that several Appalachian isolates exhibit antagonistic activity against *Escherichia coli* and *Streptococcus mutans*. These findings suggest that plant-associated bacteria from historically medicinal Appalachian flora may represent a potential source of novel biotherapeutic agents and warrant further investigation for their possible application in the prevention or treatment of bacterial wound infections. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

31. Establishment of an Avian Model of Induced Thyrotoxicosis and It's Effects on Cranial Suture Osteogenesis. *Tyler P. Hill, Holly L. Vopal. Department of Biological Sciences, Hilltop Center for Biomedical Research, West Liberty University, West Liberty, WV.*

This study aimed to validate our model of induced thyrotoxicosis and to assess differential gene expression in crania following exposure to excess thyroid hormone (TH) during embryonic development. The coronal and sagittal sutures develop from mesodermal and neural crest-derived embryonic origins, respectively. These tissues utilize both the Wnt and BMP signaling pathways. Using an avian model of induced thyrotoxicosis cranial suture tissues were collected from chicken embryos. Quantitative real-time PCR was used to quantify the expression of relevant genes. ELISA was used to quantify the levels of two proteins, osteocalcin which is primarily produced by osteoblasts, and CD34 which is a surface marker for osteoprogenitors. Based off the differential expression results from in-house PCR, cranial suture samples were submitted for NGS. Following thyroxine exposure, qRT-PCR from coronal suture samples showed no change in SMAD1 ($p=0.387$), decreased TWIST ($p<0.05$), decreased RUNX2 ($p<0.05$), increased SOST ($p<0.05$). In the sagittal suture there was a decrease in SMAD1 ($p<0.01$), no change in TWIST ($p=0.4816$), increased RUNX2 ($p<0.001$), decreased SOST ($p<0.01$). Results of the ELISA showed no significant change in CD34 and an increase in osteocalcin when exposed to thyroxine in both suture types ($p<0.05$). The NGS results allowed us to generate a heatmap which displays the genes with significantly altered expression. These findings suggest that the coronal and sagittal sutures demonstrate significantly different levels of expression following thyroxine exposure in utero and are impacting genes related to cranial development and downstream translation of Wnt signaling modulators. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

32. Role of NOD-Like Receptor X-1 in Chronic Obstructive Pulmonary Disease *Macy Hilvers¹, Madison Cooper¹, Yulu Li¹, Salik Hussain¹* ¹ *Physiology, Pharmacology and Toxicology, School of Medicine, West Virginia University, Morgantown, WV*

Chronic obstructive pulmonary disease (COPD) is a major contributor to morbidity and mortality globally, highlighting the importance of developing new treatments. West Virginia has the highest COPD prevalence at 12.6%, far above the national average of 6.5%. NLRX1 is a mitochondria-localized receptor that is implicated in inflammatory, metabolic and immune responses. While initial reports described an association between NLRX1 levels and COPD severity, the underlying mechanisms remain poorly understood. We hypothesized that NLRX1 deficiency leads to heightened inflammation and a decline in lung function by modulating innate immune responses. We created a COPD model in 10-week-old C57BL6/J and NLRX1 KO mice using four-weekly intranasal exposures to 0.2 IU of PPE. Mice were euthanized one week post last exposure and bronchoalveolar lavage (BAL) was performed to determine total and differential immune cell counts and inflammatory mediator release by ELISA. Unlavaged tissue was also preserved for histology and mRNA extraction for real-time PCR analyses. Another set of mice was used to assess lung function with the FlexiVent® technique. A significant increase in BAL lymphocytes and a greater decline in lung function was observed in NLRX1KO mice compared to WT mice. Real-time PCR analyses indicated a significantly altered expression of genes associated with inflammation (TNF- α , IL-5, IL-6, IFN- γ , IL-17) oxidative stress and mitochondrial dysfunction (IREB2), epithelial integrity and tissue repair (MMP7, MMP12, DSP, ELANE), and innate epithelial protection and surfactant homeostasis (SFTPD) in KO mice compared to WT mice. Taken together, our data confirms a significant pathophysiological role for NLRX1 in COPD. (**Supported by NIH grant R01ES031253 and Bench to Bedside Pilot Grant from 5U54GM104942-08 to Salik Hussain and Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence**)

33. Biodistribution and Cardiac Safety Evaluation in Severely Immunosuppressed Mice After Intramuscular Injection of Human Myogenic Progenitor Cells. *Munachi Ibeawuchi, John Crutchley*, Marco T. Pessoa*, Liquan Cai*, Kailey Stuart*, Sandrine V. Pierre*. *Department of Biomedical Sciences, Marshall University, Huntington, WV.*

Duchenne muscular dystrophy (DMD) is a fatal X-linked genetic disorder caused by mutations in the dystrophin gene, leading to progressive skeletal and cardiac muscle degeneration. Cell-based therapies using human induced pluripotent stem cells (hiPSCs) that have been differentiated into muscle progenitor cells (MPCs) aim to replace damaged fibers and restore healthy dystrophin production. However, evaluating the biodistribution and safety of transplanted MPCs is essential due to the potential for cell migration to unintended organs, toxicity, and tumorigenicity. This study aims to evaluate the fate and safety of MPCs after administration to the tibialis anterior (TA) muscle of an immunosuppressed mouse model, testing the hypothesis that MPCs do not migrate to cardiac tissue. Following injection of MPCs into the TA muscle cardiac tissues were collected and processed for immunofluorescence staining to confirm engraftment. Human Ku80 immunofluorescence staining confirmed MPC engraftment in the TA muscle. To test for cell migration, quantitative polymerase chain reaction (qPCR) with a human-specific assay will be used. Using mouse cardiac DNA spiked with known amounts of human DNA, we have validated this assay. To evaluate cell shape, tissue morphology, and structural abnormalities, the cardiac tissues will be stained using Hematoxylin and Eosin (H&E). Body and heart weights will also be measured to assess cardiac hypertrophy. These analyses will provide preclinical evidence supporting the safety of this cell therapy for the treatment of Duchenne muscular dystrophy. **(Supported by AHA UGR: 25IAUST1377724, AHA Marshall Summer Undergraduate Research Experience; JCESOM Matching funds and NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

34. Testing the Effect of Different Doses of Active Hexose Correlated Compound (AHCC) on McCoy Cell Growth during *Chlamydia muridarum* Infection. *Gabrielle Justice, Gebreegzabher Belay, Tesfaye Belay. Bluefield State University, Bluefield, WV.*

Studies have shown that Active Hexose Correlated Compound (AHCC) is an immunostimulatory nutritional supplement widely used in the market. However, the mechanisms of action on intestinal epithelial cells and immune cells are not well defined. Moreover, the optimal concentration of AHCC for stimulating *in vitro* epithelial cell growth is unknown. The purpose of this study was to determine the optimal dose of AHCC to stimulate growth, gene expression profiles, and secretion of proinflammatory cytokines in McCoy cells. We hypothesize that healthy growth of McCoy cells and gene expression are dose-dependent with AHCC. Monolayers of McCoy cells were treated with different concentrations of AHCC (200, 100, 50, 25, 10, 0 µg/ml) and monitored for morphological changes under the microscope over 72 h of growth. Gene expression of Interleukin-1beta (IL-1b), IL-6, and tumor necrosis factor-alpha (TNF-α) was performed using quantitative polymerase chain reaction (qPCR). Cytokine secretion was determined using Enzyme-Linked Immunosorbent Assay (ELISA). McCoy cell growth was optimal in cultures treated with 10 µg/ml, compared to cells treated with higher doses. Furthermore, IL-6 and TNF-α gene expression was upregulated in cultures treated with 10 µg/ml compared to cultures treated with higher doses. Higher concentrations saw less cell growth and restricted gene expression, possibly suggesting toxicity in high concentrations of AHCC. The pattern of cytokine secretion in AHCC-treated cultures and *Chlamydia muridarum* cultures is being assessed by ELISA. The obtained preliminary data suggest that AHCC has immunomodulatory effects via epithelial cell activation, which could be helpful for further studies in signal transduction pathways. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

35. The Role of Dorsal Root Ganglion Macrophages in Regulating the Sensations of Touch and Heat. *Samantha I. Keith*†, Wen Zheng*, Terri Poling‡, Kody M. Moore*, Edwin Wan*. *Department of Microbiology, Immunology and Cell Biology, West Virginia University School of Medicine, Morgantown WV, †Shepherd University, Shepherdstown WV, ‡Rodent Behavior Core, West Virginia University School of Medicine, Morgantown WV*

Dorsal root ganglia (DRG) are collections of sensory neurons responsible for transmitting the sensation of touch, pain, and temperature from the skin to the central nervous system (CNS). DRG also contains monocyte-derived macrophages, which are specialized immune cells that sense and eliminate pathogens and damaged cells. However, whether DRG macrophages regulate sensations is not known. We hypothesize that macrophages regulate the functions of sensory neurons, and thus, the depletion of macrophages in DRG reduces sensitivity to touch and heat. To address our hypothesis, we use a CCR2-DTR (diphtheria toxin receptor) mouse strain, in which monocytes and monocyte-derived macrophages are depleted by diphtheria toxin administration. We determine the sensitivity of the mice to touch and heat 2 days, 7 days, and 21 days following diphtheria toxin administration using the Von Frey assay and hot plate assay, respectively. We anticipate that the mice will become less sensitive to touch and heat 2 days following diphtheria toxin administration, at which monocytes and macrophages are depleted. However, the mice will gradually regain these senses on days 7 and 21, by which time macrophages repopulate in the DRG. Overall, our studies will uncover a novel role of DRG macrophages in regulating sensations. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

36. Isolation and Characterization of Potential Live Biotherapeutic Products from the Greenbrier River Against Common Bacterial Pathogens. *Sofia Kelso, Dr. Matthew Williams*

Globally, bacterial infections are the second leading cause of death, accounting for roughly 1 in 8 worldwide fatalities. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* are bacteria responsible for over half of those bacterial deaths. This issue is amplified by the continuously growing threat of antibiotic resistance. In the U.S. alone, over 2.8 million infections and over 35,000 deaths every year are caused by drug-resistant bacteria. As a solution is desperately needed, the idea of live biotherapeutic products (LBPs) has become increasingly popular. LBPs are live organisms used to prevent or treat a disease and can be found in the environment, in clinical settings, and in industry. Studies have shown that the water a person drinks affects microbiota diversity in their body. Locally, the Greenbrier River is the primary source of water for most residents, and an excellent place to look for possible LBPs. In this study, 9 isolated samples were collected from the river as it flows through Greenbrier County. The isolates were then tested to determine their potential to be used as LBP antagonists for *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus mutans*, and *Serratia marcescens*. Antagonism assays were performed, and 4 isolates showed antagonism towards an array of the pathogens. Moving forward, resiliency assays and DNA extraction will be performed to determine the viability of using these isolates as LBPs. The possibility of these isolates becoming widely available LBPs is progress towards combating the threat of antibiotic resistant bacteria. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

37. Preventing Mitochondrial Dysfunction in Aging Payton Leary*, Lawrence Grover*, PhD, Werner Geldenhuys[^], PhD, Nalini Santanam, PhD, MPH* **Department of Biomedical Sciences, Marshall University, Huntington, West Virginia, [^]WVU School of Pharmacy, Morgantown, WV.*

The study we conducted focuses on how aging diseases, such as Alzheimer's disease, occur from mitochondrial dysfunction that naturally occurs as we age. The purpose of the study was to test the impact of a synthetic drug that combats mitochondrial dysfunction by promoting mitophagy. The study was conducted on C57bl6/J mice (9 months old) that were sorted into groups based on diet, sex, and whether they received the drug or vehicle. Mice were either on a Western High-Fat Diet (42% High fat) or a Normal Chow Diet for three months before the drug or vehicle was administered for an additional three months. After six months, the mice underwent cognitive testing (novel object recognition and Y-maze), followed by metabolic testing using a Comprehensive Laboratory Animal Monitoring System (CLAMS) to measure respiratory exchange ratio (RER), VO₂, and heat production. The RER and VO₂ were more efficient in mice that were on the Normal Chow Diet, compared to those consuming a high-fat diet ($p < 0.05$). The heat produced measured the number of calories the mice were burning during activity in the chamber; the heat was significantly higher in the High-Fat Diet male mice ($p < 0.05$). Postmortem tissues and blood were taken to observe how the diet affected various inflammatory markers and mitochondrial mitophagy proteins. The blood was used for a lipid profile that revealed that the mice on the High-Fat Diet had significantly ($p < 0.05$) higher levels of HDL cholesterol and glucose. The cognitive tests and mitochondrial functional studies are currently in progress. ***(The study was funded by the WV Research Trust Fund program via a gift from the Huntington Foundation, and supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

38. Characterizing the effects of NL-1 treatment on induced ferroptosis in SIM-A9 cells. Maddie Loughborough^{1,2}, Enrique M. Carrera^{1,3}, Werner Geldenhuys^{1,3}, Jason D. Huber^{1,3}. ¹West Virginia University School of Medicine, West Virginia University, WV; ²Concord University, Athens, WV; ³Department of Pharmaceutical Sciences, West Virginia University, WV.

Traumatic brain injury (TBI) occurs in approximately 69 million people per year, causing serious cognitive dysfunction, with close to 55 million people suffering from long lasting effects. TBI causes primary injury, immediate damage upon impact, and secondary injury, the subsequent progressive deterioration. Ferroptosis, a form of iron-dependent cell death through lipid peroxidation of membranes has been identified as a key pathological mechanism during secondary injury from TBI. C1SD1 (mitoNEET), an outer-membrane iron-sulfur protein that modulates iron uptake into mitochondria, plays an important role in ferroptosis regulation. A C1SD1 ligand, NL-1 has previously been shown to have neuroprotective effects against ferroptosis post TBI; however, the mechanisms for this neuroprotection are yet to be defined. This study aimed to determine if NL-1 influences ferroptosis in microglia; the brain's neuroinflammatory cells. Protein assays, cell viability, and western blot analysis were conducted to characterize ferroptotic changes of NL-1 treatment in SIM-A9 microglia exposed to RSL3 and erastin, known ferroptosis inducers. Their IC₅₀s along with the effective concentration of NL-1, were determined using MTT assays. Western blots were utilized to evaluate several treatment groups, assessing the changes in ferroptotic protein expression. Preliminary results indicate that NL-1 has no effect on cell viability in SIM-A9 cells when using RSL3 to induce ferroptosis, suggesting NL-1 does not improve microglial cell viability *in vitro*. Therefore, microglia appear to be an unlikely mediator of NL-1's neuroprotective effects. Further studies using other cell types are necessary to identify the pathways responsible for NL-1's protective effects. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

39. Phytochemical from *Rhus copallinum* extract exhibits antimicrobial activity toward ESKAPE pathogens. Kathleen Loughman*†, Claudia Ospina^Δ, and Joseph Horzempa*. *Department of Biological Sciences, West Liberty University, West Liberty, WV; †John Marshall High School, Glen Dale, WV; Department of Natural Sciences and Mathematics, ^ΔInter American University of Puerto Rico, Bayamón, PR

Antibiotic resistance is a leading contributor of the worsening public health crisis of the 21st century. Many pathogenic bacteria are resistant to available antibiotics, especially the ESKAPE pathogens (*Enterococcus* sp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). While very few new antibiotics are in development, discovery of novel antibiotic agents is imperative to combat this threat. Natural products represent a revived area of interest as a potential source for new antimicrobials. Previous screening of ethanolic extract of *Rhus copallinum* (shining, winged, or dwarf sumac) indicated that this material exhibited marked growth inhibition against the ESKAPE pathogens. This extract was separated by column chromatography, and fractions were tested to identify bioactive phytochemicals with antimicrobial activity. In a microtiter plate, bacterial cultures were treated with the fractionated material and bacterial growth was measured using a plate reader. Wells lacking treatment or containing antibiotics known to be effective at inhibiting ESKAPE pathogens were used to identify normal or inhibited growth levels, respectively. Preliminary results have identified a compound capable of reducing bacterial growth in 4/6 ESKAPE pathogens. Future investigations will involve the characterization and a detailed analysis of the antimicrobial efficacy of this compound. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

40. Evaluation of Protein Refolding in Type 2 Diabetic Heart. *Gilbeiry Marte Diaz, Comfort Olowu. Remi Nohoesu, Atefeh Razazan, Hafsat Alabere, Joshua Mougs, Mark Eminhizer, John Hollander. Division of Exercise Physiology, Mitochondria, Metabolism, & Bioenergetics, Department of Physiology, Pharmacology, & Toxicology. West Virginia University.*

The population of West Virginia carries the highest level of diabetes in the nation; 15% of the adult population is affected. Cardiovascular disease is the leading cause of death in individuals with type 2 diabetes. Diabetic cardiomyopathy (DCM) is a diabetes induced heart muscle disorder, which is heavily driven by mitochondrial dysfunction. In diabetic hearts, mitochondrial heat shock protein 70 (mthsp70) is decreased, a crucial molecular chaperone responsible for importing, unfolding, and refolding proteins in the mitochondrial matrix. Approximately 99% of mitochondrial proteins are imported from the cytosol. For proteins to enter the mitochondrial membranes, they must unfold, revert to their primary structure, pass through the mitochondrial pores, and cross the inner and outer membranes. Hence, the objectives of this study were to evaluate mitochondrial refolding proteins in diabetic mouse models and to test an approach for evaluating protein import and refolding in mthsp70 mouse models. We hypothesize that in diabetic mice, import and refolding rates would decrease compared with control mice due to reduced mthsp70 levels. To test this, cardiac mitochondria were isolated, and refolding was conducted. Our current data show that there were fewer soluble proteins (less refolding) in diabetic cardiac mitochondria compared with controls. This suggests that protein misfolding could be the likely mechanism of mitochondrial dysfunction in the diabetic heart. Future directions would be to assess how mthsp70 overexpression restores protein import and refolding in the type 2 diabetic heart. This work may support therapies that protect patients with type 2 diabetes from cardiac complications. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

41. HERMES-AI: An Interpretable AI Framework for Modeling Immune Pathway Instability During Long-Duration Human Spaceflight. Michael J. Martínez Vega¹², Mohammad Vahed, PhD³⁴, Trupti Joshi, PhD³⁵. ¹Department of Biology, University of Puerto Rico at Humacao, Humacao, PR; ²University of Puerto Rico Comprehensive Cancer Center, San Juan, PR; ³Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV; ⁴West Virginia IDeA Network of Biomedical Research Excellence, Marshall University, Huntington, WV; ⁵Translational Bioinformatics and AI Innovations Lab, Marshall University, Huntington, WV

Long-duration spaceflight exposes astronauts to microgravity, space radiation, confinement, and circadian disruption, all of which contribute to immune dysregulation. Immune dysfunction has been recognized by NASA as a major health risk for future exploration missions. Although NASA GeneLab provides extensive transcriptomic datasets, most existing studies focus on individual gene changes rather than coordinated immune pathway alterations. Our objective was to develop HERMES-AI, an interpretable computational framework for identifying conserved immune pathway signatures associated with spaceflight. Public RNA-seq datasets from NASA GeneLab/OSDR, including astronaut and rodent immune-related studies, were harmonized into a unified analysis pipeline. Unlike conventional gene-centric approaches, HERMES-AI integrates differential expression, GO, KEGG, Reactome, and GSVA/ssGSEA analyses to generate conserved pathway-level signatures that serve as biologically interpretable features for downstream artificial intelligence modeling, enabling robust cross-study comparisons and improved biological interpretability. NASA GeneLab datasets were successfully collected, curated, and integrated into a unified analysis pipeline. Differential expression and pathway enrichment analyses identified recurrent immune-related pathways involving interferon signaling, cytokine regulation, NF- κ B/TNF signaling, antigen presentation, and adaptive immune activation across multiple datasets. Compared with individual gene analyses, pathway-level signatures demonstrated greater reproducibility across independent human and rodent studies, supporting their use as robust features for interpretable AI modeling. This study demonstrates that publicly available NASA GeneLab datasets can be integrated to identify conserved immune pathway alterations associated with spaceflight. The resulting computational workflow establishes the foundation for HERMES-AI, an interpretable AI framework for immune risk assessment and future astronaut health monitoring during long-duration space missions. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.)**

42. Impact of Norepinephrine on the Gene Expression of Siderophore Genes in Starved and Non-starved *Pseudomonas aeruginosa* Isolates. Michelle Martinez¹, Emily Shupe³.

Gebreegziabher Belay², Tesfaye Belay². ¹Bluefield Middle School, Bluefield, WV, ²Bluefield State University, Bluefield, WV, ³West Virginia School of Osteopathic Medicine, Lewisburg, WV.

Pseudomonas aeruginosa is an opportunistic pathogen that severely affects immunocompromised individuals. Studies show that adding norepinephrine (NE) to a poor medium can enhance bacterial growth, but its role remains poorly defined. It is speculated that NE is probably involved in iron acquisition. It is also known that siderophore genes are important for iron acquisition. However, it is not known whether or not NE modulates the expression of siderophore genes. The purpose of this study is to determine the impact of NE on gene expression of the siderophore genes. We hypothesize that supplementing a salt and glucose medium with NE modulates the expression of iron-acquiring genes in bacteria. The genes involved in regulating iron acquisition (*PvdS*, *RegA*, *ToxA*, and *RpsLd*) are selected in our study; we expect differential gene expression in mucoid and non-mucoid isolates. We determined the effects of NE on the growth of non-mucoid and mucoid isolates and on cell starvation. Our data show that NE increased the growth of both mucoid and non-mucoid isolates suggesting that NE is required for iron harvesting from serum. Preliminary data show that isolate PAO1 had up-regulated expression of *pvdS* and *rpsLd* in cultures grown in the presence of NE. In contrast, *RpsLd* was down-regulated in mucoid isolates; however, *pvdS* and *ToxA* were up-regulated in non-mucoid isolates supplemented with NE. Overall, our results show that i) the NE has a positive growth effect on mucoid and non-mucoid isolates, and ii) the genes *rpsLd* and *ToxA* have different profiles of expression in both isolates. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

43. Antibacterial effects of *Monarda Fistulosa* (Bee Balm) nanoemulsion against drug-resistant bacterial pathogens. *Miguel Mascarenhas, Madhav Nair, and James Wilkes West Virginia School of Osteopathic Medicine, Lewisburg, WV*

With the increasing incidence of antibiotic resistance in bacterial pathogens, it is critical to identify alternative therapeutics to combat infections. Essential oils have been investigated as a potential option due to their natural antimicrobial compounds. The essential oil nanoemulsions are a potential delivery mechanism for the therapeutic. In this study, we investigated the antimicrobial properties of *Monarda Fistulosa* (Bee Balm) Oil. Due to its concentrations of carvacrol and thymol, this oil has been effective at breaking down bacterial cell walls and destroying motility structures, thereby preventing the growth of these microbes. In this study, bee balm essential oil nanoemulsions were prepared from varying concentrations of bee balm, tween 80, and water. These nanoemulsions were then tested using various assays, including the Disk Diffusion Assay, Minimum Inhibitory Concentration, and Minimum Bactericidal Concentration, to assess antibacterial activity. The assays demonstrated the antibacterial effect of bee balm oil against a variety of bacterial species in a concentration-dependent manner. Overall, this study demonstrated that more research is needed into the mechanism of action of how bee balm essential oil inhibits different types of bacteria and if these results can be used to develop a wound dressing or wound bandage that could potentially be used to treat various bacterial infections. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

44. Caffeine Effects on Locomotor Activity of Madagascar Hissing Cockroaches. *Isadora Matthews**, *Katharine Birkman**, *Ruth Conley**, *Qing Wang#*. **Department of Natural and Physical Sciences, Shepherd University, Shepherdstown, WV; #Department of Computer Science, Mathematics and Engineering, Shepherd University, Shepherdstown, WV.*

The effects of caffeine on the locomotor activity of the Madagascar hissing cockroach (*Gromphadorhina portentosa*) have not been well documented. Although caffeine is a well-known stimulant that influences nervous system activity and locomotion in both humans and many insect species, its effects on Madagascar hissing cockroaches remain largely unexplored. This study investigated the impact of caffeine exposure on the locomotor behavior of these cockroaches. Madagascar hissing cockroaches were first dehydrated and their baseline locomotor activity was recorded. Individuals were then assigned to one of three caffeine treatment groups with different concentrations or to a control group. Following treatment, locomotor activity was monitored for 10 minutes in a behavioral chamber. The percentage of time spent moving and movement speed were quantified using Python-based motion-tracking software. Preliminary results indicate that cockroaches exposed to both low and high concentrations of caffeine spent a greater percentage of time moving than those in the control group. In addition, individuals exposed to the highest caffeine concentration exhibited more than a twofold increase in movement speed after two days of exposure. Overall, caffeine-treated cockroaches displayed increased locomotor activity compared with untreated controls. Data collection is ongoing to increase the sample size and improve statistical power. Upon completion of the study, one-way ANOVA followed by Tukey's post hoc test will be used to determine whether differences in locomotor activity among treatment groups are statistically significant. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

45. Restoration of Immune Responses in a β 2-Adrenergic Receptor-Deficient Stress Murine Model during *Chlamydia muridarum* Genital Tract Infection. Robert McClain², Gebreegzabher Belay¹ Tesfaye Belay³. ¹Bluefield State University, Bluefield, WV ²Woodrow High School, Beckley, WV.

The relationship between stress, chlamydia genital disease, and its influence on the host immune response remains unknown. Nerve cells produce stress hormones such as norepinephrine, which suppresses immune function by binding to the β 2-adrenergic receptor (β 2-AR), but the mechanism of suppression remains unknown. This study analyzed the immune responses in a stressed β 2-AR knockout (KO) mouse model compared with those in a stressed wild-type (WT) C57BL/6J mouse. We hypothesized that norepinephrine receptor deficiency leads to a recovery of immune response in stressed mice. Cytokine production, gene expression profiles of transcription factors, and expression of major surface markers on CD4⁺ T cells in stressed KO and WT mice were measured by ELISA, qPCR, and flow cytometry, respectively. Differential cytokine production was observed; however, in general, restoration of Th1, Th2, Th17, and regulatory T cell cytokine production was observed in stressed β 2-AR KO mice compared to stressed WT mice. Similarly, differences in the gene expression of the T-bet, retinoic acid-related orphan receptor γ t (ROR γ t), and FoxP3 transcription factors in stressed *C. muridarum*-infected mice were identified. Increased gene expression of ROR γ t and FoxP3, and interleukin-17 and interleukin-10 production in stressed mice, indicated upregulation of Treg cells in stressed mice. However, gel electrophoresis of qPCR end products for transcription factors showed no significant differences in DNA intensity between groups. Restoration of expression of major CD4⁺ T cell surface markers was observed. The data from *C. muridarum* genital infection in the mouse model may provide insights into preventing and controlling chlamydia in humans during stressful conditions. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

46. KAP1 Cooperates with HIF Signalling to Regulate Hypoxia-Driven Gene Expression in Breast Cancer. *Millar McElrea, Dr Alexey Ivanov. Department of Biology-Chemistry, University of Charleston, Charleston, WV; Department of Biochemistry and Molecular Medicine, West Virginia University School of Medicine, Morgantown, WV.*

Hypoxia, or reduced oxygen availability, is a defining feature of many solid tumours and promotes breast cancer progression by activating hypoxia-inducible factors (HIFs). HIF transcription factors function as heterodimers composed of a constitutively expressed β subunit and an oxygen-regulated α subunit, and they regulate genes involved in cell survival, metabolic adaptation, angiogenesis, invasion, and metastasis. The transcriptional regulator KAP1/TRIM28 has been shown to interact with and cooperate with HIF1 α ; however, its broader role in HIF-mediated transcriptional signalling remains poorly understood. This study investigates how KAP1 cooperates with HIF transcription factors to regulate gene expression under normoxic and hypoxic conditions in breast cancer cells. Using shRNA-based approaches, we generated MDA-MB-231 breast cancer cell lines with stable single and double knockdown of HIF subunits, including HIF1 α , HIF2 α , HIF1 β /ARNT, HIF2 β /ARNT2, and KAP1. To define the domains responsible for KAP1-HIF interactions, we will perform co-immunoprecipitation and Western blot analysis using KAP1 deletion mutants together with individual HIF subunits. In parallel, luciferase reporter assays are being used to assess KAP1-dependent regulation of HIF1 β and HIF2 β promoter activity. Complementary studies in HEK293T cell models expressing wild-type or mutant KAP1 proteins are evaluating the functional KAP1 domains required for hypoxia-induced gene regulation. Preliminary progress includes the successful generation and validation of multiple knockdown cell lines, as well as establishment of experimental systems for protein-interaction and transcriptional-reporter analyses. Defining the molecular relationship between KAP1 and HIF signalling may reveal previously unrecognized mechanisms of hypoxia adaptation and metastatic progression, providing insight into potential therapeutic targets for aggressive breast cancer. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

47. Effects of Exercise and Vaping on Adolescent Behavior. Katherine Meek, Nick Williams, Elaina Blickenstaff, Samuel Tetteh-Quarshie, Lawrence Grover, Brandon Henderson, Nalini Santanam. Department of Biomedical Sciences, Marshall University Joan C. Edwards School of Medicine, Huntington, WV.

Vaping is currently popular among adolescents. Research shows flavoring in e-cigarettes can have similar negative effects to nicotine, even when nicotine is absent. This is mainly due to increased oxidative stress. Exercise and catalase are known to reduce oxidative stress. Still, their effectiveness on the impact of vaping has not been studied. This study aimed to observe the effectiveness of exercise and catalase on mitigating the harmful effects of nicotine and flavoring, independently, in e-cigarettes. 6 groups of adolescent-aged (4-5 weeks old) C57bl6 or Catalase-transgenic male mice: (i) sedentary, (ii) nicotine, (iii) green apple (GA), and their exercise counterparts were used. Over eight-weeks, mice were exposed to nicotine (6 mg/mL) or green-apple flavoring (15 mg/mL) for 2 hr/day, 5 days/week. Exercised mice ran 5 days/week at 15 m/min. Open field (anxiety) behaviors and grip strength were observed every two weeks. At the end of the eight weeks, CLAMS and lipid profile were performed. C57/nicotine/exercise mice had the highest increase in HDL; C57/GA/exercise mice had the highest decrease in triglycerides. Catalase mice had more consistent HDL and TRG levels. Catalase/nicotine/exercise mice had more improved grip strength than catalase/GA/exercise. Both groups of catalase/nicotine mice had lower edge-times and higher center-times than the C57 mice, whereas both groups of GA mice had higher edge-times (more anxious). Catalase/GA mice showed more improvement in center-time with exercise than nicotine mice. Catalase/nicotine mice had significantly lower VO₂ than C57/nicotine mice; this switched with the incorporation of exercise. Catalase and exercise improved behavior in vaping adolescent mice. **(Supported by AHA UGR: 25IAUST1377724, AHA Marshall Summer Undergraduate Research Experience; JCESOM Matching funds and NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

48. Assessing Caffeine Preference and Addiction-Like Feeding Behavior in Madagascar Hissing Cockroaches (*Gromphadorhina portentosa*) Using a Two-Choice Preference Arena. Juan J. Mercado-Trinidad*, Ruth Conley**, Qing Wang#. *Spring Mills High School, Martinsburg, WV; **Department of Natural and Physical Sciences, Shepherd University, Shepherdstown, WV; #Department of Computer Science, Mathematics and Engineering, Shepherd University, Shepherdstown, WV

Hands-on laboratory investigations provide an effective approach for engaging high school students in authentic scientific inquiry while reinforcing concepts in neuroscience, psychology, and experimental design. This proposed study aims to develop and evaluate an inquiry-based laboratory activity that investigates addiction-like feeding behavior in Madagascar hissing cockroaches (*Gromphadorhina portentosa*) using a two-choice preference arena. *G. portentosa* was selected as the model organism due to its simple nervous systems, ease of care, and suitability for classroom environments. Twenty cockroaches will be assigned to either a control group or a caffeine treatment group (2.0 mg/mL) and observed over a 7-day experimental period consisting of a baseline preference phase followed by chronic caffeine exposure. Behavioral responses will be quantified by measuring food consumption, time spent near each food source, frequency of visits, and a calculated Preference Index to assess changes in food preference over time. The experimental design incorporates standardized environmental conditions and randomized food placement to minimize bias and demonstrate principles of controlled experimentation. Beyond investigating the effects of chronic caffeine exposure on feeding behavior, this laboratory activity is designed to provide high school students with opportunities to formulate hypotheses, collect and analyze quantitative data, interpret behavioral patterns, and apply statistical reasoning. The proposed investigation offers an accessible, low-cost model for integrating neuroscience, psychology, animal behavior, and scientific inquiry into secondary science curricula while promoting data literacy and evidence-based thinking. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

49. Selection of *Neisseria gonorrhoeae* mutants resistant to resorufin morpholinoethyl ether. Rebekkah Merinar¹, Alex Tarr¹, Mackenzie Hall¹, Randall A. Koziel², Vahid Lotfikalajahi², Brian V. Popp², Hacer Karatas Bristow², and Deanna M. Schmitt¹, ¹Department of Biological Sciences, West Liberty University, West Liberty, WV; ²C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV

Neisseria gonorrhoeae is a Gram-negative bacterium responsible for the sexually transmitted infection gonorrhea, which is becoming increasingly difficult to treat due to rising antibiotic resistance. Resazurin-based compounds, such as resorufin morpholinoethyl ether (R-mor), have shown promise as antimicrobial agents against *N. gonorrhoeae*. However, the mechanism of action of these compounds is not known. Given that a common strategy bacteria employ to develop antibiotic resistance is altering the drug's target, we sought to select for R-mor-resistant isolates and use reverse genetics to elucidate the mode of action of resazomycins. To select for R-mor resistant isolates, we cultured the *N. gonorrhoeae* strain FA1090 in progressively higher concentrations of R-mor. Over 20 passages, we were able to successfully culture the strains in the presence of 22 µg/mL R-mor, a concentration 16-fold higher than the minimal inhibitory concentration (MIC) of this compound. To compare the R-mor MIC of these resistant isolates to wild type FA1090, a modified broth microdilution assay was performed. Genomic DNA was then extracted from these resistant strains and will be sent for sequencing to identify potential genetic mutations associated with resistance. While the sequencing results are pending, this work provides insights into how *N. gonorrhoeae* adapts to R-mor and lays the groundwork for future studies to optimize or overcome resistance to resazurin-based antibiotics. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

50. Effects of Macromolecular Crowding on the Kinetics of Pyruvate Kinase Muscle 1 with Variable ADP and PEP Substrates *Jannah N. Miller, Rafe M. Riley, Kelsey A. Aldrich* Department of Chemistry and Biochemistry, West Virginia Wesleyan College, Buckhannon, West Virginia

The interiors of cells are crowded, and can contain a macromolecule concentration of 30-40% w/v. However, in vitro enzymatic assays are commonly carried out in dilute solution, despite growing bodies of research indicating that the kinetic parameters of enzymes are significantly impacted when assessed in crowded solutions more akin to those present in cells. In this work, the ADP and PEP dependent kinetic parameters of human pyruvate kinase muscle isoform 1 (hPKM1) were experimentally determined under crowded conditions using the sugar polymer Ficoll and the protein bovine serum albumin (BSA) as model macromolecular crowders in lactate dehydrogenase (LDH)-coupled assays. hPKM1 was selected for assessment primarily owing to its indispensable place in the glycolysis pathway and overall metabolism of the energy-intensive tissues in which it resides. It was observed that increasing macromolecule concentrations did not significantly impact K_m or catalytic efficiency in both ADP and PEP-dependent kinetics. Notably, k_{cat} was significantly increased under BSA crowding conditions, but significantly decreased under Ficoll crowding conditions when PEP is the variable substrate; this trend was not observed when the concentration of ADP is varied. Overall, increased macromolecule concentrations do not significantly alter the kinetic properties of hPKM1, highlighting that hPKM1 is one instance of an enzyme not affected by high macromolecule concentrations. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

51. Synovium-Associated Stem Cells: Comparative Biology of Synovium-Derived and Infrapatellar Fat Pad-Derived Stem Cells as Superior Sources for Cartilage Regeneration.

Catherine A. Millman†, Ming Pei. † West Virginia IDeA Network for Biomedical Research Excellence, Sumer Research Program, Morgantown, West Virginia; Tissue Engineering Laboratory, Division of Exercise Physiology, Department of Orthopedics, West Virginia University, Morgantown, West Virginia*

Articular cartilage is damaged in osteoarthritis (OA), a condition that primarily affects older adults. Because cartilage has limited intrinsic regenerative capacity, and this capacity declines further with age, healing is especially difficult. Stem cell-based approaches offer a promising alternative. Previous studies identified synovium-derived stem cells (SDSCs) as a viable option for cartilage regeneration, and later work showed that the infrapatellar fat pad also contains a distinct population of stem cells. The objective of this review is to compare SDSCs and infrapatellar fat pad-derived stem cells (IPFPSCs) across several factors to determine which source is superior for cartilage regeneration and repair. SDSCs and IPFPSCs were found to share similar molecular profiles, both expressing Sox9, Runx2, TGF- β , Wnt, BMP, Aggrecan, and Type II Collagen. SDSCs showed better proliferation and chondrogenic differentiation when treated with a combination of IGF-I, FGF-2, and TGF- β 1. IPFPSCs, on the other hand, offer easier surgical access, lower donor-site morbidity, and retain chondrogenic potential even in OA-affected joints. Both cell types demonstrated immunomodulatory and paracrine activity that supports cartilage repair. In conclusion, SDSCs appear biologically superior for chondrogenic regeneration, while IPFPSCs may be more advantageous for clinical use. These findings suggest that synovium-associated stem cells represent a promising option for cartilage repair. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

52. Increased Keratin 18 Expression Suppresses Brain Metastatic Potential of Breast Cancer

Cells. Abigail Mills, OMS II ¹; William Liu, OMS II ¹; Maggie Maguire, OMS II ¹; David Brooks, OMS II ¹; Christopher Butler, BS ¹; Gabor Szalai, PhD ²; Anissa Johnson, DO ³; Garrett Clemons, PhD ¹; Travis Salisbury, PhD ⁴; James Denvir, PhD ⁴; Paul Lockman, PhD ⁵; Tuoan Liu, MD, PhD ¹. 1. Department of Biomedical Sciences, West Virginia School of Osteopathic Medicine, Lewisburg, West Virginia, United States of America. 2. Department of Biomedical Sciences, Burrell College of Osteopathic Medicine, Las Cruces, New Mexico, United States of America. 3. School of Medicine, Loma Linda University, Loma Linda, California, United States of America. 4. Genomics and Bioinformatics Core Facilities, Marshall University, Huntington, West Virginia, United States of America. 5. Department of Pharmaceutical Sciences, College of Pharmacy, West Virginia University, Morgantown, West Virginia, United States of America.

Triple-negative breast cancer (TNBC) provides a unique challenge for treatment as the markers typically targeted are absent. The prognosis for TNBC patients with brain metastasis is especially poor, thus it is important to understand its mechanism and develop new therapeutics for treatment. We previously reported that expression of the keratin 18 gene, an epithelial marker, is decreased in brain metastasis breast cancer cells (231Br) compared to their parental 231 cells. The hypomethylating agent azacitidine (AZA) was shown to decrease brain metastasis in both *in vitro* cell and *in vivo* mouse models. We also confirmed that keratin 18 is less expressed in metastatic compared to primary breast cancer patients. Therefore, we hypothesize that keratin 18 regulates brain metastasis and AZA sensitivity. To test this, we established two stable cell lines (231Br+keratin 18 overexpressing keratin 18 and 231Br+GFP), characterized the property of metastasis, and tested AZA efficacy between them. Our results showed that the 231Br+keratin 18 cells have decreased migration and invasion potential. Additionally, treating both cell lines with AZA induced significantly less apoptosis in 231Br+keratin18 than 231Br+GFP cells. AZA was also shown to less effectively inhibit cell growth, cell migration and invasion potential, Wnt signaling, expression of metastasis-related proteins, and VEGF release in 231Br+keratin 18 cells. In conclusion, keratin 18 overexpression decreases the growth, migration, and invasion of brain metastatic TNBC cells, and reduces their response to AZA treatment. Therefore, keratin 18 plays an important role in regulating brain metastasis and may be a potential drug target for treatment. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

53. Monocarboxylate Transporter 2 (MCT2) Mitigates Lung Tumor Progression by Shaping the Immune Microenvironment Duyen Nguyen ¹, Yen On Chan ^{2,3}, Zhuanhong Qiao ⁴, Trupti Joshi ^{2,3,5,6}, David Gozal ⁴ and Abdelnaby Khalyfa ^{2*}Department of Biomedical Sciences, University of Charleston, Charleston, WV 25304. Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, 25755. Christopher S Bond Life Science Center, University of Missouri-Columbia, MO, 65201. Department of Pediatrics, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, 25755. MU Institute for Data Science and Informatics, University of Missouri-Columbia, MO, 65201. Department of Biomedical Informatics, Biostatistics, and Medical Epidemiology, School of Medicine, University of Missouri-Columbia, MO, 65201.

The SLC16A7 gene encodes the monocarboxylate transporter 2 (MCT2), a high-affinity pyruvate transporter with emerging roles in cancer metabolism. Previous studies have shown that deletion of MCT2 promotes tumor growth in lung cancer, suggesting a tumor-suppressive function; however, the cellular mechanisms through which MCT2 modulates the tumor microenvironment (TME) remain poorly understood. Single-nucleus RNA sequencing was performed on TC1 murine lung tumors from three MCT2 knockout (KO) and three control (CO) mice, yielding 18,859 nuclei after quality control. Data were processed using the Seurat pipeline with SCTransform normalization and Harmony integration then clustering into distinct populations. Population-wise differentially expressed genes were utilized with PanglaoDB to annotate clusters into 8 distinct cell types. Differential expression analysis was then performed between KO and CO within each cell type, followed by KEGG pathway enrichment analysis using clusterProfiler. Cell type annotation indicated a heterogeneous TME including macrophages, B cells, memory T cells, endothelial, fibroblasts, ependymal cells, and neutrophils. KEGG pathway enrichment analysis in macrophages, the biggest immunological population in this study, revealed considerable enrichment in MAPK, PI3K-Akt, and IgSF CAM signaling pathways. Key genes include inflammatory signaling regulators (Map3k14), cell survival and extracellular matrix-related genes (Spp1, Igf1r), and cell adhesion molecules (Slit2). This suggests enhanced inflammatory activity, macrophage activation, and cell-cell interactions within the TME. These findings indicate that MCT2 loss broadly reshapes TME by modulating macrophage-associated signaling networks and may contribute to lung cancer progression, thereby identifying prospective therapeutic targets for lung cancer treatment. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

54. Na⁺/K⁺-ATPase Modulation with Ouabain Induces Protection of Human Induced Pluripotent Stem Cell-Derived Cardiac Progenitor Cells Against Hypoxia: Role of Metabolic Preconditioning.

Zackary D. Nunley¹, Sandrine V. Pierre^{2,3}, Marco T. Pessoa^{2,3}.¹Department of Chemistry, Marshall University, Huntington, WV; ²Department of Biomedical Sciences, Marshall University, Huntington, WV; ³Joan C. Edwards School of Medicine, Huntington, WV.

Poor survival of transplanted cardiac progenitor cells (CPCs) limits the efficacy of stem cell-based therapies for myocardial infarction, creating a need for strategies that enhance cellular resilience. The Na⁺/K⁺-ATPase (NKA) regulates ionic homeostasis and activates signaling pathways that modulate cellular metabolism. Low concentrations of the cardiotonic steroid ouabain (OUA) can stimulate NKA signaling without inhibiting ion transport, but the effects on CPC protection remain unclear. We hypothesized that low dose OUA induces metabolic preconditioning through NKA signaling, enhancing CPC resistance to hypoxic injury. CPCs were differentiated from human induced pluripotent stem cells and characterized by decreased expression of pluripotency markers (*NANOG* and *OCT4*) and increased expression of CPC markers (*ISL1* and *NKX2.5*). Immunocytochemistry for *ISL1* and *NKX2.5* confirmed a highly enriched CPC population. To assess cellular protection, CPCs were pretreated with 1-10 nM OUA for 24h before exposure to hypoxia (1% O₂) for 30h. Cellular injury was quantified by measuring lactate dehydrogenase (LDH) release into the culture medium. OUA significantly reduced LDH release by approximately 40% compared with untreated controls (p<0.05). Mitochondrial respiration was evaluated using the Seahorse XFp Analyzer and demonstrated that pretreatment with 10 nM OUA increased maximal respiration and spare respiratory capacity. These findings suggest that low dose OUA induces metabolic preconditioning that enhances CPC resistance to hypoxic stress, supporting a cytoprotective role for NKA-mediated signaling. Targeting NKA-dependent metabolic preconditioning may represent a novel strategy to improve CPC survival, engraftment, and therapeutic efficacy following transplantation into the infarcted myocardium. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

55. Investigating the Effects of NL-1 on Induced Ferroptosis in IMA2.1 Cell Line *Ridwan Oloyede^{1,2}, Enrique M. Carrera^{1,3}, Dr. Werner Geldenhuys^{1,3}, Jason D. Huber^{1,3}. ¹West Virginia University School of Medicine, West Virginia University, WV; ²Glenville State University, Glenville, WV; ³Department of Pharmaceutical Sciences, West Virginia University, WV.*

Ischemic stroke, the most common type of stroke, is a leading cause of death worldwide and lacks effective neuroprotective therapies. Treating this condition is complicated by reperfusion injury, as it can induce several neurodegenerative pathologies, one of which that has gained recent attention is ferroptosis. Ferroptosis is a form of cell death caused by iron-accumulation in the cell, inducing lipid peroxidation and production of reactive oxygen species (ROS). MitoNEET Ligand 1 (NL-1) is a therapeutic compound that binds to mitoNEET (or CISD1), a mitochondrial outer membrane protein regulating iron transport, and has demonstrated neuroprotective properties. Research has shown that NL-1 is effective against ferroptosis, but the mechanism of its neuroprotective effects and specific cells affected remain unknown. In the brain, astrocytes ensure the proper functioning of neuronal cells and maintaining neuronal homeostasis following ischemic injury, making them a potential therapeutic target. This project was focused on investigating effects of NL-1 in astrocytes experiencing ferroptosis. Ferroptosis was induced using RSL3 and Erastin, which inhibit Glutathione Peroxidase 4 (GPX4) and promote lipid peroxidation. The IC₅₀s for both compounds and the effective concentration of NL-1 needed to restore function were determined using MTT assays. Western blotting was run to evaluate protein expression, and GSH/GSSG assay was run to measure oxidative stress in cells. Results show that NL-1 does not significantly increase astrocyte cell viability when treated with Erastin. Future studies will further characterise and understand the interaction between NL-1 and astrocytes in its therapeutic role against ferroptosis. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

56. Characterizing the Contribution of Histamine on Cardiomyocyte Hypertrophy. *Marin Parker, Heather Linsky, Kallie Shaffner, Alexander Widiapradja, Scott Levick.*

Cardiac hypertrophy is a major risk factor for heart failure. Histamine, a biogenic amine produced primarily by mast cells, has been previously reported to be upregulated in patients with coronary artery disease and has been linked to cardiac hypertrophy. Clinical association studies investigating histamine-2 receptor blockade indicate positive outcomes on blood pressure, heart rate, and overall heart failure status, suggesting that this receptor may promote hypertrophy. The present study aimed to assess direct actions of histamine on cardiomyocyte hypertrophy. We hypothesized that histamine would induce cardiomyocyte hypertrophy via histamine-2 receptor activation. H9c2 cells (ATCC #CRL-1446), a ventricular myoblast cell line derived from embryonic rat hearts were differentiated to a cardiomyocyte-like cell type using retinoic acid (1 μ M) for 5 days followed by treatment as follows: 1) untreated, 2) histamine (10 μ M) for 24 hours (n=6/group). The histamine-2 receptor was identified on H9c2 cells using immunofluorescence. H9c2 cells were assessed for hypertrophy via DNA/protein ratio and fetal gene (Nppa, Nppb, Myh7) expression using qPCR. The results from this study indicated no significant changes in hypertrophic measures upon stimulus with histamine. Herein, we conclude that histamine does not exert direct effects on hypertrophy in a H9c2 cardiomyocyte-like cell. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

57. Putting the “Pro” in Probiotics: How Microorganisms are Changing the Playbook Sagar S. Patel, MBS, OMS-II*, Matthew Williams, PhD*. West Virginia School of Osteopathic Medicine, Lewisburg, WV

Probiotics are live microorganisms that provide health benefits when introduced into a host. They help maintain a balanced microbiome through supporting immune function and nutrient absorption. Most probiotics on the market are anaerobic and include *Bifidobacterium* and *Lactobacillus*. In athletics, probiotics are effective in endurance-based and intermittent exercise. They help athletes strengthen training and promote faster recovery by increasing skeletal muscle mass and reducing inflammatory markers. The gut microbiome plays an active role in sports, as studies have shown that regular exercise significantly reduces the abundance of pathogens such as *Clostridium difficile*. Chicory is a plant used as a prebiotic and often added to coffee. Chicory is beneficial to humans by promoting butyrate-producing and fiber-degrading bacteria. Butyrate is a short-chain fatty acid that helps to maintain the anaerobic environment in the gut and enhances muscle turnover. To analyze the ability of chicory to antagonize human pathogens, multiple bacterial strains were isolated from chicory. Isolates were analyzed for their effects against pathogenic bacteria. Additionally, we evaluated the role of multiple *Bacillus* species against pathogens. The *Bacillus* genus helps break down fiber and maintain a low-oxygen environment in the gut by promoting aerobic metabolism. We found that three isolates from chicory were able to antagonize our panel of human pathogens. With our *Bacillus* studies, we found that *Bacillus pumilis* was the most promising probiotic candidate. Future steps include utilizing different agar plates to visualize fiber breakdown and butyrate production to determine if these are important mechanisms of action of these bacteria. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

58. Ferroptosis pathway and Pterostilbene Attenuation of Hydrogen Peroxide (H₂O₂)

Cytotoxicity in Human Renal Proximal Tubular Epithelial (HK-2) Cells. Seth Perry#, Kathleen C. Brown*, Elizabeth McGuffey*, Christopher Bender* and Monica Valentovic*. #Hurricane High School, Hurricane, WV; *Department of Biomedical Sciences, Toxicology Research Cluster, Joan C. Edwards School of Medicine Marshall University, Huntington, WV.

Clinical studies have reported beneficial effects of pterostilbene (PTER) in patients with cancer, hepatic and renal disease. PTER is a stilbene analogue of resveratrol that has a beneficial property of higher bioavailability, and both agents may reduce or delay the development of renal damage in patients undergoing cancer chemotherapy treatment or being exposed to situations that increase reactive oxygen species (ROS) levels. The goal of this study was to probe the beneficial effects of PTER in human proximal tubular epithelial cells exposed to H₂O₂, a ROS associated with cisplatin nephrotoxicity. HK-2 cells were selected as these cells are a noncancer human proximal tubular (PT) epithelial cell line. The hypothesis of this project was that PTER preserved glutathione peroxidase (GPX4) which would protect renal cells from damage mediated by the ferroptosis pathway. HK-2 cells were plated and equilibrated 48h followed by a 1h pretreatment with 0-10 uM PTER followed by 24h co-incubation with H₂O₂. A minimum of 3 independent experiments were conducted with different cell passages. Upon completion of the treatments, cells were collected for evaluating cell cytotoxicity using the MTT assay conversion to formazan. Cells were collected after the incubation period and lysed for western blot analysis. GPX4 and SLC7A11 were measured as biomarkers of the ferroptosis pathway. GPX4 protein expression was not reduced in the presence of PTER with or without H₂O₂. In summary, PTER protective effects on H₂O₂ was associated with maintaining GPX4 protein expression which would reduce ferroptosis mediated cell damage. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence and NIH Grant U54GM104942-08)**

59. Structure-Activity Relationship (SAR) studies identify DOHEVANIL as a potent, non-pungent growth-suppressive agent in human adenosquamous carcinoma (ASC-L). *Joeusel A. Ramos Torres¹, Aliyah N. Webb², Sarah D. Wooten³, Sarah L. Miles³, and Piyali Dasgupta³. ¹University of Puerto Rico at Mayagüez, ²MTTC, ³Department of Biomedical Sciences, Marshall University, Huntington, WV*

Adenosquamous carcinoma of the lung (ASC) is a rare subtype of non-small cell lung cancer (NSCLC) that exhibits features of both adenocarcinoma (gland-forming) and squamous cell (flat cell) carcinoma within the same tumor. ASC-Ls are characterized by rapid doubling time, aggressive clinical course, and a dismal survival rate. The long-term objective of our laboratory is to identify dietary natural compounds that could suppress the growth of human lung cancers. Our published data show that capsaicin (the spicy ingredient of chili peppers) robustly suppresses the growth of human ASC-Ls. However, the clinical development of capsaicin as a viable anti-cancer drug is hampered by its adverse side effect profile. One strategy to circumvent the unfavorable side effects of capsaicin is to develop non-pungent capsaicin analogs with potent growth-inhibitory activity. The structure of capsaicin can be divided into three distinct domains, Regions A, B, and C. We undertook an extensive SAR analysis to identify non-pungent capsaicin analogs that would display robust inhibition of cell viability. We screened Region A, B, and C analogs using MTT assays on the human ASC-L cell line H647. Our experiments identified the non-pungent capsaicin analog DOHEVANIL as a potent growth-inhibitory agent in human ASC-Ls. Our future studies will examine the growth-suppressive activity of DOH in ASC-L organoids and in mice models **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

60. Localization of the divisome protein FtsL in *Francisella tularensis* LVS. Abigale Riggle and Stuart Cantlay, Department of Biological Sciences, West Liberty University, West Liberty, WV, 26074.

Francisella tularensis is a gram-negative bacterium categorized as a tier 1 select agent. Fatal disease can be caused by very low doses, leading to the potential of *F. tularensis* being used as a bioterror agent. Understanding cell division in *F. tularensis* could yield important insights for the development of a vaccine or antimicrobial treatments. Understanding how *F. tularensis* divides may also shed light on how the bacterium is able to enter a Viable-but-not-Culturable (VBNC) state. This VBNC state could be a contributing factor to survival in the environment and during phagocytosis into host cells. Known divisome proteins of *Escherichia coli* include FtsZ, FtsA, and ZipA, which are involved in the assembly of the cytokinetic proto-ring. Divisome proteins, such as the FtsQLBWI complex and FtsN are recruited to the proto-ring, directing the formation of the division septum. Currently, very little is known about the *F. tularensis* divisome. Ftl_1540 is predicted to encode the FtsL homolog in *F. tularensis* Live Vaccine Strain (LVS). To learn more about the function of FtsL, we have generated the fluorescence fusion protein, FtsL-EMGFP. Cloning Ftl_1540 into two EMGFP reporter plasmids, pSC18, containing a strong promoter, and pSC13, containing no promoter, has resulted in the two recombinant plasmids pAR2 and pAR1, respectively. Recombinant plasmid pAR2 is suspected to express FtsL-EMGFP, which we aim to localize in bacterial cells, while pAR1 serves as a negative control. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

61. Investigating Myogenic Regulation of Craniofacial Morphology in a Avian Model of Craniosynostosis. *Olivia M. Rocchio, Emily K. Stotts, Tyler P. Hill, Dr. Nicole Garrison, and Dr. Holly L. Vopal. Department of Biological Sciences, Hilltop Center for Biomedical Research, West Liberty University, West Liberty, WV*

Craniosynostosis, the premature fusion of cranial sutures, is a clinical manifestation and has more recently been established as a risk factor associated with maternal thyrotoxicosis. Thus far, our research has focused on the mechanism of excess thyroid hormone (thyroxine, T₄) acting directly on bone cells in the regulation of osteogenesis. However, our preliminary transcriptomic analysis of collected cranial sutures from our embryonic avian model of induced thyrotoxicosis suggested a potential bone-muscle crosstalk. Gene set enrichment indicated an upregulation of muscle-related genes including ACTC1, MYOD1, ACTA1, and MYOZ2; as well as osteogenic and Wnt/ β -catenin signaling genes such as LRP6. These results suggest that excess T₄ may alter muscle progenitor cells in addition to bone-associated cells. We hypothesize that thyroxine-induced Wnt/ β -catenin signaling contributes to the differential expression of bone- and muscle-associated proteins in the coronal suture. Both muscle-related and Wnt/ β -catenin signaling proteins were analyzed from collected coronal sutures by Western Blotting. Results indicated that β -catenin expression increased with treatment alongside increased negative feedback regulator sclerostin, indicated elevated Wnt signaling. However, no significant differences in ACTC1 and ACTA1 were observed, despite the increase in the transcription of the gene. Future studies will further investigate the potential crosstalk between muscle and bone cells in the coronal suture. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

62. The Effects of Vaping on Liver extracellular vesicles from adolescent mice. *John Rose, Jady Simon, Samuel Tetteh-Quarshie, Brandon Henderson, and Nalini Santanam. Department of Biomedical Sciences, Marshall University, Huntington, West Virginia.*

Vaping, the current alternative to cigarette smoking, exposes the body to many different toxins and chemicals. This can cause damage to many organs, such as the liver, which filters toxins and chemicals in the body's bloodstream. The damage caused by vaping on the liver is currently not known and was the focus of this study. Previously, our laboratory showed that vaping resulted in increased oxidative damage to the liver. In the current study, we proposed to test if vaping altered liver extracellular vesicles and their cargo. To measure this effect, liver tissues from six groups of adolescent mice (n=4-6/group) sedentary, exercise, nicotine, nicotine plus exercise, green apple flavor, green apple flavor plus exercise were used. Small extracellular vesicles (sEVs) were isolated from the liver using the ultracentrifugation method. Nanoparticle Tracking Analysis (NTA) was used to determine particle size and concentration of sEVs. Additionally, miRNA array and oxidative stress marker genes were performed to profile the specific vesicular cargo. From NTA analysis, it was determined that there was no statistical significance between the particle size and concentration across all groups. However, it did suggest a higher concentration of sEVs for the group with green apple flavoring plus exercise. This is likely due to a physiological response to the combined effects of the chemical stress of the green apple flavor with the metabolic demands of exercise. This higher concentration of sEVs in these tissue samples may induce a higher level of oxidative stress in liver cells. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

63. Active Hexose Correlated Compound Enhances Dendritic Cell Stimulation in Stressed Mice during *Chlamydia muridarum* Genital Infection. *Jaida Rottenbery, Gebreegziabher Belay Tesfaye Belay. Bluefield State University, Bluefield, WV.*

Dendritic cells (DCs) are known to present AHCC to T cells in the lymph node; however, their specific functions in our stress model remain undefined. The purpose of this study was to explore the role of DCs by determining cytokine production in DCs and T cells. We hypothesize that AHCC feeding to stressed mice enhances the gene expression and secretion of proinflammatory cytokines in DCs and T cells. AHCC-fed BALB/c mice were stressed 5 minutes daily for 21 days and infected with *C. muridarum* intravaginally. After 7 days of infection, bone marrow macrophages (BMDs) and mature intestinal DCs were purified, cultured, and RNA was isolated for gene expression analysis. Culture supernatants were collected to determine cytokine production using ELISA. Gene expression of interleukin-1beta (IL-1 β), IL-6, and Tumor Necrosis Factor-alpha (TNF- α), major surface marker expression on DCs, and signature surface marker profiles were determined by quantitative polymerase chain reaction (qPCR) and flow cytometry, respectively. ELISA results show that AHCC increases interleukin. 12, interferon-gamma (IFN- γ), and TNF- α production by DCs compared with stressed PBS-fed mice. Gene expression of proinflammatory cytokines showed inconsistent results, likely due to RNA isolation quality. Higher expression of DC surface markers was obtained in AHCC-fed mice compared to PBS-fed stressed mice. Overall, AHCC feeding increases the production of protective and suppressive cytokines by DCs. The obtained preliminary data suggest that AHCC has immunomodulatory effects via immune cell activation, including DCs, which could be helpful for further studies in stressed physiological animal and human subjects. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

64. Establishment of Human iPSC-Derived Skeletal Muscle Cells to Investigate Na⁺/K⁺-ATPase Signaling During Muscle Regeneration. *James A. Scott, Sandrine V. Pierre, Liquan Cai, Department of Biomedical Sciences; Joan C. Edwards School of Medicine, Marshall University, Huntington, WV*

Millions of people worldwide are affected by chronic kidney disease (CKD), a progressive disorder associated with skeletal muscle wasting and impaired muscle regeneration. Previous research suggests that Na⁺/K⁺-ATPase functions not only as an ion transporter but also as a signaling molecule involved in skeletal muscle physiology. Altered Na⁺/K⁺-ATPase signaling has been implicated in CKD; however, its role in muscle regeneration remains incompletely understood. Human induced pluripotent stem cell (hiPSC)-derived and human satellite cell-derived skeletal muscle cell cultures provide a physiologically relevant in vitro model to investigate these mechanisms. The objective of this study was to establish and maintain hiPSC-derived skeletal muscle cultures and evaluate the effects of pharmacological modulation of Na⁺/K⁺-ATPase signaling using ouabain, a ligand for the Na/K ATPase receptor. Human hiPSC-derived and satellite cell-derived skeletal muscle cultures were maintained under sterile conditions and treated with vehicle control or 1 nM, 2 nM, and 4 nM ouabain. Cell morphology, attachment, viability, and proliferation were monitored by routine microscopy. Total RNA was isolated from treated cultures for downstream quantitative PCR (qPCR) analysis of Na⁺/K⁺-ATPase-related genes and skeletal muscle regeneration markers. Successful establishment and maintenance of proliferating skeletal muscle cultures were achieved. Preliminary observations demonstrated robust attachment and growth under control conditions, whereas ouabain-treated cultures exhibited concentration-dependent changes in morphology and growth characteristics, suggesting altered Na⁺/K⁺-ATPase signaling. Ongoing qPCR analyses will determine whether these morphological changes are accompanied by altered expression of genes involved in Na/K ATPase signaling and myogenesis. This work establishes a human cell-based platform for investigating Na⁺/K⁺-ATPase signaling and provides a foundation for understanding mechanisms contributing to CKD-associated sarcopenia. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

65. Toward Precision Radiation Therapy Through Quantitative EPR Oximetry *Ganga P. Sharma¹, Benoit Driesschaert^{2,3}* ¹*Department of Natural Sciences, Fairmont State University* ²*WVU Cancer Institute Research Programs, WVU Cancer Institute* ³*Department of Pharmaceutical Sciences, WVU School of Pharmacy*

Tumor oxygenation plays a critical role in radiation therapy outcomes, with hypoxic tumor regions demonstrating enhanced radioresistance that can compromise treatment effectiveness. Accurate and quantitative measurement of oxygen levels is therefore essential for developing oxygen-guided strategies to improve therapeutic outcomes. This study investigates quantitative electron paramagnetic resonance (EPR) oximetry using the oxygen-sensitive probe dFT-PFP dissolved in perfluorocarbon solvents for precise oxygen measurement. Oxygen-dependent changes in EPR linewidth were characterized under controlled oxygen environments to establish calibration relationships between molecular oxygen concentration and spectral response. The perfluorocarbon-based system provides a highly oxygen-permeable and chemically stable environment, enabling sensitive detection of oxygen fluctuations through EPR spectroscopy. These calibration studies establish a foundation for applying dFT-PFP-based EPR oximetry toward noninvasive assessment of tumor oxygenation and improved radiation treatment planning. By enabling quantitative characterization of the tumor oxygen landscape, this approach has the potential to support personalized radiotherapy strategies that account for biological variations in tumor oxygen status. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

66. A Genetic Rescue Model to Investigate the Therapeutic Window for Degenerating Cones

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Blue cone monochromacy (BCM) is an X-linked retinal disease caused by deletion or missense mutations in the L- and M-cone opsin genes, resulting in severely reduced or absent L/M-cone function while S-cone and rod-mediated vision remain intact. Critically, affected cones retain structurally intact inner segments, making them viable targets for gene therapy. DKO and C198R mouse models faithfully phenocopy BCM, exhibiting shortened cone outer segments and absent cone function, with over 50% of cones retaining intact inner segments through at least five months of age. AAV-mediated gene therapy effectively rescues cone function in younger mice, but efficacy declines significantly by five months despite substantial cone survival — raising the question of whether this reflects AAV delivery limitations or intrinsic age-dependent changes in cone rescue capacity. To address this, we generated a tamoxifen-inducible genetic rescue system that bypasses AAV delivery entirely. Opn1mw knock-in mice carrying a stop sequence in intron 1 were crossed with Pde6g-Cre mice; tamoxifen-induced Cre-mediated recombination removes the stop cassette and restores endogenous opsin expression. Opn1mw hemizygous males showed absent M-cone ERG responses with normal S-cone function, and tamoxifen administration restored cone function and opsin expression, validating the system. Tamoxifen will now be administered at five months and at terminal degeneration stages to precisely define the therapeutic rescue window. If cones remain rescuable at advanced ages, the observed decline in AAV efficacy points to delivery limitations rather than cone-intrinsic resistance, with important implications for broadening the treatment window in BCM and other cone-predominant diseases. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

67. Thyroxine Exposure Enhances Late Stage Osteoblast Maturation in Cranial Sutures From Embryonic Chickens Using an Avian Model of Induced Thyrotoxicosis. *Emily K. Stotts, Olivia M. Rocchio, Tyler P. Hill, Dr. Holly L. Vopal. Department of Biological Sciences, Hilltop Center for Biomedical Research, West Liberty University.*

Thyroid hormone (TH) plays an important role in regulating growth, development, and metabolism of cells in the body. However, thyrotoxicosis, an excess amount of TH circulating in the body can alter normal tissue development, especially in pregnant women. During pregnancy, uncontrolled thyrotoxicosis can cause several birth defects; including craniosynostosis (CS), the premature fusion of one or more of the cranial sutures on the infant's skull. Approximately 3% of all CS cases are linked to maternal thyrotoxicosis, but the direct mechanism behind TH-induced CS is still widely unknown. However, there are several phases in the maturation of osteoblasts, or bone-building cells, where TH could be interfering with normal development. To mimic maternal thyrotoxicosis, fertilized chicken eggs were injected with 50ng thyroxine. Samples of the coronal and sagittal sutures were taken for protein analysis. Markers for early-stage osteoblasts (CD34 and RUNX2); late-stage osteoblasts (Osteocalcin); and Wnt signaling (β -Catenin, Axin-2, and Sclerostin), were measured via ELISA and/or Western Blotting. Additionally, crania were fixed to perform double-antibody immunofluorescent whole-mount staining to visualize the presence of development markers LMX1B and RUNX2. It was expected that by inducing thyrotoxicosis, the rate of osteoblast commitment and maturation would increase. Osteocalcin, Sclerostin, β -Catenin, and Axin-2 were significantly upregulated ($p < 0.05$) in coronal sutures with treatment. However, there was no significant difference between CD34 and RUNX2. Indicating that excess T₄ has a more significant impact on mature osteoblasts by hyperactivation of Wnt signaling and encouraging the promotion of bone mineralization in coronal sutures. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

68. The Effects of Circulating Extracellular Vesicles on Endothelial Progenitor Cell Function Under Hypertensive Conditions. *Madison Stratton, Shuzhen Chen, Jinju Wang. Department of Biomedical Sciences, John C. Edwards School of Medicine, Huntington, WV*

Hypertension is a major factor in cardiovascular disease. It is commonly associated with impaired vascular repair and endothelial dysfunction. Circulating extracellular vesicles (cEVs) are mediators. These mediators may also contribute to vascular dysfunction by altering the endothelial progenitor cell (EPC) function through their molecular cargo. The study investigated whether hypertension alters the functions of circulating extracellular vesicles, while also evaluating their role in regulating EPC function. cEVs were isolated from the plasma of wild type (WT) mice and transgenic hypertensive mice (Alb1) and denoted as WT-cEVs and HTN-cEVs. EVs were characterized by nanoparticle tracking analysis (NTA) for size and concentration and Western blot analysis with EV markers. The functions of cEVs were tested on a co-culture system with human EPCs. Cellular EV uptake, migration and proliferation capabilities were detected. MicroRNA (miR) was extracted from cEVs, and RNA-Seq was conducted for miR cargo analysis. There is no difference between the size and concentration between the WT-cEVs and HTN-cEVs. Western blot confirmed the presence of EV markers CD81 and TSG101. Uptake of both WT and hypertensive cEVs by EPCs were demonstrated. Additionally, HTN-cEVs reduced migration capability of EPCs, while had no effect on the proliferation of EPCs as detected by Ki67 staining. RNA-Seq identified miR profiling alteration in HTN-cEVs as indicated with 13 miRs were downregulated and 11 miRs were upregulated. The data suggests that HTN-cEVs reduced the migration capability of EPCs. The study will continue to investigate the alternations of miR in cEV cargo contributes to EPC function change under hypertensive conditions. (**Supported by AHA UGR: 25IAUST1377724, AHA Marshall Summer Undergraduate Research Experience; JCESOM Matching funds, AHA-B-CDA (JW), and WVCTSI pilot grant (JW), and NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence**)

69. Pyruvate Kinase L is Sensitive to Physiological Macromolecule Concentrations. Addison P. Taylor, Mary L. Genes, Kelsey A. Aldrich. Department of Chemistry and Biochemistry, West Virginia Wesleyan College, Buckhannon, West Virginia

About 30-40% of a cell's volume is composed of macromolecules, altering the behavior and functions of enzymes that operate within the crowded environment through entropic and enthalpic effects. However, most *in-vitro* experiments do not account for physiological macromolecular concentrations, producing results that may not align with typical cellular activity. The limited research examining the changes in enzymatic activity with physiological macromolecule concentrations suggest that the effects are highly enzyme specific. We experimented with liver pyruvate kinase (PKL), the isoform primarily located within the liver and kidneys, which maintains the vital function of catalyzing the final, irreversible step of glycolysis. Previous research indicates that PKL is affected by macromolecular crowding; taking this into account, we performed LDH-coupled assays with bovine serum albumin (BSA) and Ficoll as model crowders in varying concentrations. Kinetics with ADP as the variable substrate resulted in minimal changes in K_m values and differential effects on V_{max} . However, when PEP is the variable substrate with BSA as the model crowder, there is an increase in the V_{max} values and a large decrease in the K_m values, resulting in a 42-fold increase in catalytic efficiency. Ficoll, however, did not have an extreme effect on PEP V_{max} and K_m values. The importance of exploring both substrates within a bisubstrate enzyme and multiple macromolecular crowders should be considered, as a trend or effect within the data values of one substrate may not be reflected in the other substrate or other model crowders. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

70. Early Neurobehavioral Effects of Prenatal Nano-Titanium Dioxide Exposure in Adult Sprague-Dawley Rats. *Sabrina Thorn¹, Teresa Gluth¹, Taryn Veltri¹, Landen Bach¹, Sarah Keeley¹, Jacob W. Feldmann¹, Terri Poling², James Walton², Elizabeth C. Bowdridge¹. West Virginia University Department of Physiology, Pharmacology, & Toxicology and Department of Neuroscience.*

Nano-titanium dioxide (nano-TiO₂) is a widely used whitening agent commonly found in plastics, sunscreen, toothpaste, and food products. Utilization of nano-TiO₂ spanning across multiple industries increases opportunities for human exposure, including during pregnancy. Perinatal exposure to nano-TiO₂ caused long-term neurobehavioral impairments in adult rodents. We sought to assess the consequences of prenatal nano-TiO₂ exposure on neurodevelopmental behaviors, including general locomotor activity, exploratory drive, anxiety-like actions, and spatial memory in adult Sprague-Dawley F1 progeny. Pregnant dams were assigned to filtered air (control; n=9) or nano-TiO₂ (n=6), and behavioral testing was conducted in F1 offspring (control males, n=15; control females, n=23; nano-TiO₂ males, n=15; nano-TiO₂ females, n=6). Neurological assessment and Open Field testing began at 10 weeks postnatally, with Y-Maze testing at 13 weeks postnatally. Exposed female offspring demonstrated significantly increased locomotor activity (p=0.023), whereas there were no significant differences in exposed male offspring. Exposure had no effect on central tendency, rearing, or change in central tendency across intervals in males; however, there was a trend in central tendency over time in exposed females (p=0.09). Additionally, exposed females exhibited significantly increased spontaneous alternation (p=0.001), while exposed males showed no significant difference. Taken altogether, these results reveal that prenatal nano-TiO₂ exposure causes early, sex-specific neurobehavioral changes in exploratory and locomotor behavior, but not anxiety-like behaviors or spatial working memory in exposed females. Results support that neurobehavioral deficits due to prenatal nano-TiO₂ exposure can be identified during early development and support continued evaluation to determine whether these alterations persist or increase with age. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

71. Comparison of two pre-clinical AAV gene therapy vectors in mouse models of blue cone monochromacy. *Mary E. Tracey, Brooke A. Brothers, Madison Ashcraft, Qingyan Wang, Saya Geisendorfer, Wen-Tao Deng. Dept. of Ophthalmology / Dept. of Biochemistry & Molecular Medicine, West Virginia University.*

Blue cone monochromacy (BCM) is an inherited retinal disease in which mutations in the OPN1LW/OPN1MW gene cluster cause the red and green cones in the retina not to function properly. BCM patients display severely reduced visual acuity, loss of color vision, and daylight blindness. The goal of this project is to compare the rescue efficacy and levels of transgene expression of two preclinical AAV vectors for BCM. Both AAVs contain the same promoter and transgene for human L-opsin. However, the two AAVs differ in the capsids they are packaged and the routes that they are delivered. The two AAVs being tested are AAV.SPR which is subretinally delivered and AAV.7m8 which is injected intravitreally. It is predicted that the AAV.SPR will show higher expression in the cones but cause more damage to the retina due to subretinal injections, while AAV.7m8 will show lower expression but cause less damage during the injection. Two mouse models were used to represent the two most common BCM mutations in humans, a double knockout mouse for the deletion mutations, and a C198R mouse for the corresponding human C203R missense mutation. The two AAVs were injected in both mouse models and Electroretinography (ERG) were recorded at 1 and 2 months post-injections. We showed that AAV.SPR by subretinal injections robustly rescued cone photoreceptor function at both time points assessed in both mouse models, while both mouse models injected by intravitreal injections of AAV.7m8 showed no ERG rescue. Our data has important implications for moving BCM into pre-clinical stage. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

72. A Method for Extracting and Purifying and Modifying, 1 mg Quantities of Sialylglycopeptide for Capillary Electrophoresis Assays. Emerson Tracy¹, Tolulope Betty Akingbaso², Eric Corley², Daniel Oladuga², Lisa Holland². ¹Department of Shepherd University, Shepherdstown, WV. ²C. Eugene Bennett Chemistry Department, West Virginia University, Morgantown.

N-Glycans play an important role in host-pathogen interactions, making them valuable targets for biomedical research. However, N-glycan standards are costly. Therefore, developing a cost-effective way to obtain them will advance biomedical research. Sialylglycopeptide contains a disialylated complex biantennary N-glycan and can be further modified to obtain a pure N-glycan. The high commercial cost (\$2,569.80 for 20 mg sialylglycopeptide) limits its use as an N-glycan source. This research adapted previously reported methods to extract sialylglycopeptide efficiently from eggs. Crude sialylglycopeptide was obtained by removing the lipids and proteins in eggs and concentrating the sialylglycopeptide in 40% acetone. The sialylglycopeptide was then purified using a carbon/celite extraction followed by purification with a disposable phenylboronic acid solid phase extraction cartridge. The purified sample was quantified using capillary electrophoresis to evaluate the recovery. The N-glycan was cleaved from the sialylglycopeptide and further quantified. The N-glycan standard can be further modified using benchtop enzymatic modification methods. The N-glycan standard obtained with this work is applied to influenza A H1N1 neuraminidase enzyme assays. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

73. Differences in DNA Methylation Profiles Associated with HPV Status Suggest Disruptions in Cancer Associated Pathways. Shayna M. Tysor¹, Juliana M. Serrano-Rodríguez^{2,3}, Jurelis Torres-Reyes^{2,4}, Jeannette L. Salgado-Montilla², Gabriel Borges-Vélez², and Josué Pérez-Santiago^{2,4}
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Human papilloma virus (HPV) is the most common sexually transmitted infection in the world, in which high-risk genotypes are responsible for over 70% of oral cancers. People with HIV (HIV) have a higher risk for HPV-related cancers with limited early identification markers capable of detecting oral early oncogenesis. Epigenetic changes in the DNA, such as methylation, can be a molecular marker of cancer as it can change gene transcription and therefore influence cancer initiation and progression. In this study, we analyzed methylation differences in relation to HPV status. We collected saliva from 56 PWH (33 HPV+ vs 23 HPV-) and evaluated DNA methylation patterns using the Illumina Infinium MethylationEPIC Array. We identified 1,766 differentially methylated positions (DMPs, p-value < 0.05) in which 64% of DMPs were hypermethylated and 36% were hypomethylated in HPV+ subjects. The DMPs were mostly located within the gene bodies, where 422 probes (~23%) were hypermethylated and 275 (~15%) probes were hypomethylated. Functional analysis identified biological processes including cell migration, extracellular matrix organization, signaling by Rho GTPases, and intracellular signaling significantly associated with HPV. Protein-protein interaction networks identified HDAC1 (which corresponds to a DMP) as one significantly changed protein. It has been related to cancer as an epigenetic regulator, primarily controlling histone deacetylases. Differences in these DNA methylation markers may suggest a potential role of epigenomic reprogramming in the natural history of HPV and may represent novel early identification markers for oral oncogenesis. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

74. Sex-Specific Cognitive Effects of Prenatal Nano-TiO₂ Exposure and Preventive Febuxostat Treatment. Taryn G. Veltri*, Teresa D. Gluth†, Sabrina L. Thorn†, Terri J. Poling†, Elizabeth C. Bowdridge†. *Department of Biology and Environmental Sciences, West Virginia Wesleyan College, WV; †Department of Physiology, Pharmacology, and Toxicology, West Virginia University, WV

Nanomaterials have a variety of applications, such as medicine, consumer products, and industry, causing widespread human exposure. Maternal nano-titanium dioxide (nTiO₂) inhalation exposure has been shown to cause motivation deficits, working and short-term memory impairments, and slower decision-making capabilities in male Sprague-Dawley rat offspring. There is currently little to no research regarding cognitive effects of maternal inhalation on female offspring as well as potential prenatal interventions. We hypothesize that prenatal nTiO₂ exposure will result in sexually dimorphic effects on cognitive performance. Additionally, we propose that providing the mother with Febuxostat, an FDA-approved xanthine oxidase inhibitor, during pregnancy will protect offspring from nTiO₂-induced cognitive deficits into adulthood through decreased oxidative stress and improved uterine blood flow. Pregnant rats were randomly assigned into four groups and given the following treatments: 1) Air and water, 2) Air and Febuxostat, 3) nTiO₂ and water, and 4) nTiO₂ and Febuxostat. Locomotor activity and anxiety-like behavior were tested in the offspring at 18-weeks-old using an open field test. Results showed a statistically significant difference ($p < 0.0001$) in total movement percentage between the female air and nTiO₂ offspring suggesting hyperactivity. Furthermore, spatial working memory was tested with a Y-maze at 20-weeks-old. The male nTiO₂ and water group demonstrated significantly fewer spontaneous alternations than both the air plus water group ($p < 0.0481$) and the nTiO₂ plus Febuxostat group ($p < 0.0336$), suggesting inhibited spatial working memory in males exposed to nTiO₂ and not treated with Febuxostat. Considering this, Febuxostat warrants further investigation as a potential prenatal intervention to reduce cognitive deficits into adulthood. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

75. Sensitizing BRCA-Proficient Triple-Negative Breast Cancer Brain Metastasis to PARP Inhibition Through Arginine Deprivation *Breanna Waldron¹, Olivia Carpenter-Milam¹, Cullen Wolford¹, Geoffrey Pecar^{1,2}, Paul R. Lockman¹* Department of Basic Pharmaceutical Sciences, School of Pharmacy, West Virginia University (HSC), 64 Medical Center Dr., Morgantown, WV 26505 MD/PhD Program, School of Medicine, West Virginia University (HSC), 64 Medical Center Dr., Morgantown, WV 26505

Brain metastases (BrM) will develop in 10-16% of breast cancer patients. Treatment options include surgical resection, radiation, and systemic therapies. A limitation of systemic treatment efficacy is the blood-brain barrier (BBB), which controls what exits and enters brain and limits drug accumulation, leading to a dismal survival of 4-16 months. In presence of BrM, the BBB becomes heterogeneously leaky, creating the blood-tumor barrier, yet it continues to limit drug accumulation at therapeutic concentrations. Therefore, discovering novel therapeutic approaches is critical. A hallmark of cancer is metabolic rewiring, including arginine (Arg) auxotrophy, characterized by the inability to produce Arg endogenously. Depriving cells of Arg induces DNA damage and downregulates critical homologous repair (HR) proteins leading to HR deficiency (HRD). PARP inhibition is a viable option for patients with HRD. We hypothesize reducing Arg in BRCA-proficient triple-negative breast cancer (TNBC) cells and inhibiting PARP to target HRD will enhance cell death. Arg auxotrophy and DNA repair deficiency was assessed using western blotting and immunofluorescence imaging. MTS assays were performed to determine cell viability following Arg reduction and niraparib treatment at various time points (24hr, 48hr, and 72hr). Arg metabolic enzymes ornithine transcarbamylase and argininosuccinate synthase 1 were reduced in MDA-MB-231 cells, but not in 4T1 cells. There were differences in DNA repair proteins (RAD51D, FANCG, PARP) following Arg reduction compared to control. Cells showed lower cell viability when treated with reduced Arg and Niraparib. Experiments suggest Arg reduction sensitizes cells to Niraparib, potentially leading to a promising strategy for TNBC BrM treatment. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

76. Inhibition of SARS-CoV-2 3CL protease (NSP5) Activity with mucosal IgA antibodies *Taytum Walls, Meenal Elliott, Timmy Nguyen*

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, enters the host through the respiratory tract, making mucosal immunity an important first line of defense against infection. While current COVID-19 vaccines primarily target the viral spike protein, host immune responses against non-structural proteins, which are essential for viral replication, have received comparatively little attention. The focus of this research is to evaluate whether secretory IgA antibodies found in human saliva can recognize the viral main protease NSP5 and determine whether it can inhibit its proteolytic activity. Twenty saliva samples collected from healthy individuals with a recent SARS-CoV-2 infection were evaluated for their ability to inhibit cleavage of a canonical target sequence (SILQG) within the viral polypeptide by His-tagged 3CLpro using a fluorescence resonance energy transfer (FRET) assay. Cleavage of host cadherins CDH6 and CDH12 containing the target sequence SILQG was examined using a gel-based assay with His-tagged 3CLpro. FRET-based assay demonstrated that saliva IgA inhibited the proteolytic activity of His-tagged 3CLpro. These findings support the potential role of salivary IgA in limiting NSP5 proteolytic activity and provide a foundation for further studies using an air-liquid interface mucosal model for viral infections. In summary, IgA antibodies in the normal immune response against non-structural proteins of SARS-CoV-2 likely contribute to effective viral clearance in healthy individuals. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence and Research Challenge Grant No. HEPC.DSR.18.6 from the Division of Science and 437 Research, WV Higher Education Policy Commission, and NIH/NIGMS Award Number 2U54GM104942-07)**

77. The non-pungent capsaicin analog DOHEVANIL triggers ferroptosis in human endometrioid ovarian carcinoma (EOC) cells. Aliyah N. Webb¹, Joeusel Torres Ramos², Sarah D. Wooten³, Sarah L. Miles³ and Piyali Dasgupta³. ¹BridgeValley Community, and Technical College, Charleston, WV, ²University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico, ³Department of Biomedical Sciences, Marshall University, Huntington, WV

Endometrioid ovarian carcinoma (EOC) of the ovary represents a distinct histological subtype of epithelial ovarian cancers, and account for 10%–20% of all ovarian malignancies. The development of EOCs is often associated with endometriosis. The long-term objective of our laboratory is to identify nutrition-based agents relevant for the treatment of EOCs. Specifically, our laboratory explores the anti-cancer activity of capsaicin (the hot and spicy ingredient of chili peppers in human EOCs. Using structure activity relationship studies, we have identified DOHEVANIL as a non-pungent capsaicin analog which displays robust growth-inhibitory activity in human EOCs. The primary aim of my summer research project was to identify the molecular mechanisms underlying the growth-suppressive activity of DOH in human EOC cells. We performed MTT assays to evaluate the signaling pathways underlying DOH-induced decrease of cell viability in human EOC cells. We observed that the treatment of human A27890 EOC cells with DOH in the presence of the ferroptosis inhibitor ferrostatin -1 abrogated the growth-hindering activity of DOH in human EOC cells. We repeated the MTT experiments with a second distinct inhibitor of Ferroptosis (namely Liproxstatin-1) and obtained similar results. We confirmed these findings by performing a caspase-3 activity apoptosis assay. We found that both ferrostatin-1 and liproxstatin-1 reversed the pro-apoptotic activity of DOH in EOC cells. Our future studies include the measurement of specific ferroptosis biomarkers and measurement of intracellular iron load in DOH-treated EOC cells. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

78. Endometriosis and Cardiovascular Health: An understudied connection. Samiya Webb, Jadyn Simon, Harshal Sawant. Brenda Mitchell, MD, Nalini Santanam, PhD, MPH

Endometriosis (EM) is a disease of the reproductive system where the endometrial tissue grows outside of the uterus, typically on other pelvic organs or, in rare cases, distant areas like the lungs or brain. It affects 10% of childbearing-age women, can cause infertility and severe pain, with no known etiology. Current treatments are ineffective, and it is linked to a higher risk of cancer and other chronic conditions, such as cardiovascular disease (CVD). We aim to identify the cellular factors that are transformed during EM and subsequently become risk factors for CVD. We hypothesize that circulating small extracellular vesicles (sEVs) are bioactive factors that alter cardiac and vascular function in EM. Circulating sEVs were isolated from blood obtained from women with and without EM (n=6). The size and number of sEVs were determined using NTA. MiRNA cargo of the sEVs was determined using PCR arrays. The effect of sEVs on endothelial function was determined using a wound assay. NTA of circulating sEVs from EM patients showed higher size and lower concentrations compared to Control patients. The endothelial cell scratch assays using sEVs from the EM patients displayed delayed wound healing rates compared to those from the control group (20% vs 50% closure in 48 hours, EM vs Control). sEV cargo showed nine miRNAs that were differentially expressed in EM compared to Ctrl groups, which might play a role in endothelial dysfunction (miR-320b, miR-200a/b). This study suggests that the circulating sEVs from EM compared to Control patients promote endothelial dysfunction. ***(Supported by AHA UGR: 25IAUST1377724, AHA Marshall Summer Undergraduate Research Experience; JCESOM Matching funds and NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence).***

79. Genetic Differentiation of Nutritionally Deprived *Pseudomonas aeruginosa*. Daron Weekley*, Tesfaye Belay† and Stuart Cantlay*. *Dept of Biomedical Sciences, West Liberty University, WV, 26074. † Bluefield State University, Bluefield, W.V. 24701.

Pseudomonas aeruginosa is an opportunistic bacterium known for its high level of resistance to antibiotics and disinfectants, which contributes to increased morbidity and mortality following infection. One well-studied context of *P. aeruginosa* infection is cystic fibrosis, where the bacterium forms persistent biofilms in the lungs. *P. aeruginosa* ATCC 39324 is commonly used to investigate biofilm formation and antimicrobial resistance mechanisms. Preliminary analysis of an existing transcriptomics data set comparing starved vs non-starved *P. aeruginosa* ATCC 39324 was performed using the EPI2ME Labs wf-transcriptomes workflow, comparing RNA reads from each sample to the well-characterized *P. aeruginosa* PAO1 reference genome. This analysis found that genes related in pyocyanin production were upregulated initially, then downregulated after a nutritional downshift in the non-starved samples after one week. Conversely, pyocyanin production was initially downregulated in starved samples, then upregulated after one week of nutritional downshift. These findings suggest potential transcriptional differences between long-term starved and non-starved *P. aeruginosa*. To understand more about these differences, *P. aeruginosa* ATCC 39324 that had been subjected to long-term starvation was compared to a non-starved wild-type ATCC 39324 culture. Starved and non-starved specimens were sampled at multiple time points following reintroduction to growth conditions: initial, 15 minutes, 30 minutes, 3 hours, 24 hours, and 1 week. Total RNA was extracted from each time point for sequencing using Oxford Nanopore Technologies. Additionally, crystal violet assays were performed to analyze the effects of starvation on biofilm formation. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence and was made possible by funding from NASA West Virginia Space Grant Consortium).**

80. Fate-mapping Pseudohypoxic Cells in Triple-Negative Breast Cancer *Hannah Wheaton**, *Elena Pugacheva†* **Department of Natural and Physical Sciences, Shepherd University, Shepherdstown WV †WVU Cancer Institute, West Virginia University, Morgantown WV*

Triple-negative breast cancer (TNBC) accounts for ~10-15% of all breast cancer diagnoses but accounts for a disproportionately high percentage of deaths (~40%). Because TNBCs lack the three cell surface markers used in standard breast cancer tests, diagnosis and treatment are challenging. Therefore, early detection and prevention of cancer spreading (metastasis) is crucial for curative treatment of TNBCs. Hypoxia promotes metastasis in breast cancer. One of the regulators of HIF1 is Aurora Kinase A (AurkA). Our lab previously reported that nuclear-localized AurkA is associated with activation of HIF1, leading to systemic dissemination of cancer cells from mammary tumors even under oxygenated conditions (pseudohypoxia), and subsequent metastases outgrowth. We hypothesize that HIF-AurkA activity leads to the early dissemination of TNBC cells. Pseudohypoxic cells induced by AurkA activate different sets of genes than hypoxia (low oxygen, 1%), and therefore require different treatment approaches. To differentiate between oxygenated and hypoxic cells based on HIF1 activity, we engineered TNBC cells with hypoxia-inducible fluorescent reporters. The reporter design allows us to detect fluorescence only in cells exposed to low oxygen but not in control oxygenated cells (O₂, 5-19%). The fluorescent signal is permanent and will stain cells even if they are reoxygenated later, thus enabling us to trace the lineage of the resulting metastases after injection of these cells into the mammary glands of mice to model a spontaneous metastatic cascade. The developed methodology will enable us to discover new therapeutic targets that drive early dissemination and have the potential to improve outcomes for TNBC patients. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

81. Determining Gene Expression Changes Induced by Ganoderic Acid A in Estrogen Receptor Positive and Estrogen Receptor Negative Human Breast Cancer Cells. *Claire Wheeler*[†], *Travis Salisbury*^{*}, *Mohit Verma*^{*}. ^{*}*Marshall University Joan C. Edwards School of Medicine, Huntington, WV;* [†]*West Virginia Wesleyan College, Buckhannon, WV*

Breast cancer (BC) is the second most common cancer diagnosis and second leading cause of cancer death in women. Ganoderic Acid A (GAA) has been shown to decrease cell proliferation, although the mechanism is unknown. Based on previous studies, we hypothesize GAA regulates the expression of genes involved in cell proliferation. Estrogen receptor positive (ER+) MCF7 and ER- MDA-MB-231 breast cancer cells were treated with either vehicle (DMSO) or GAA for 24 hours. Total RNA was extracted using Qiagen RNA purification columns, Illumina-compatible libraries were constructed, and samples were sequenced on a NextSeq2000 Illumina Sequencer at approximately 20 million paired end reads per sample. FASTQ reads underwent quality control, adapter trimming, alignment to the human reference genome, gene counting, differential expression analysis, and pathway enrichment analysis. RNA-Seq showed 8000 genes differentially expressed between MCF7 and MDA-MB-231 cells. GAA had a weak effect on gene expression in MCF7 cells, with only nine genes significantly changed relative to vehicle. MDA-MB-231 cells were more responsive, with approximately 30 upregulated and 40 downregulated genes. Pathway enrichment analysis showed gene expression changes were significantly associated with biological pathways involved in cell adhesion, and filament-regulated processes. In addition to these pathways, genes regulated by GAA are involved in cell proliferation and cytoskeletal organization, suggesting potential mechanisms behind its decrease in cell proliferation effects in BC cells. **(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)**

82. Development of novel biotherapeutics for the treatment of opportunistic pathogens in humans. *Garon Wiseman, Matthew Williams*

Around the world, approximately one in every eight deaths is caused by some form of bacterial infection. In 2019, 7.7 million deaths were attributed to bacterial infections, and of these deaths, around 5 million were caused by bacterial infections resistant to antibiotics. To combat this, the expansion of biotherapeutic discovery and use has been seen as a possible solution. In this experiment, isolates from varying sources have been used to investigate antagonism against five different human pathogens: *Escherichia coli*, *Klebsiella Pneumoniae*, *Staphylococcus Aureus*, *Streptococcus Mutans*, & *Serratia Marcescens*. Isolates were harvested from plant life local to southern West Virginia (chicory & pawpaw) and were run in spot/antagonism assays against the pathogens. If positive results were shown, then further testing, such as supernatant assays, was done on said isolates and pathogens. Multiple isolates have been found thus far in the research and show promising capabilities to be used in biotherapeutics, and further research is being done to investigate the mechanisms of their antagonism. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***

83. Reprogramming of Branched-Chain Amino Acid Metabolism in Breast Cancer *Nevaeh Ann Ellington, Emine C. Koc, Hasan Koc*

Remodeling of energy metabolism is described as one of the major hallmarks of cancer, contributing to its heterogeneity and survival in a dynamic environment with reduced nutrient and oxygen levels. Metabolic pathways leading to energy generation by oxidative phosphorylation (OXPHOS) play crucial roles in supporting high-energy demands in cancer, specifically the essential branched-chain amino acids (BCAAs), valine, leucine, and isoleucine. The BCAAs imported into mitochondria naturally become an important part of mitochondrial energy metabolism. They have already been implicated in diseases including cancer, aging, and other metabolic disorders. In this study, secondary analysis of the mass spectrometry-based proteomics was performed on breast cancer tumor tissues and cell lines, along with the immunoblotting analyses of triple-negative and ER/PR(+) cell lines and tissue biopsy lysates. The impaired expression of BCAA catabolic enzymes, BCAT1 and BCKDH complex subunits, was clearly shown in both tumor tissues and cell lines and helped explain the remodeling of OXPHOS in specific subtypes of breast cancer. Changes in expression of BCAA catabolic enzymes could be developed to characterize the heterogeneity of energy metabolism in breast cancer and develop diagnostic and prognostic markers to determine aggressive subtypes in breast cancer, and may provide novel therapeutic possibilities. ***(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)***