PRINBRE
IDeA Network of Biomedical Research Excellence

SUMMER STUDENT RESEARCH SYMPOSIUM 2025

VOICES OF DISCOVERY: STUDENT RESEARCH PRESENTATIONS ACROSS PARTNER INSTITUTIONS AND PROGRAMS.

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ABSTRACT SUPPLEMENT

University of Puerto Rico/MD Anderson Cancer Center
US4 Partnership for Excellence in Cancer Research
RESEARCH EDUCATION CORE

VOICES OF DISCOVERY: STUDENT RESEARCH PRESENTATIONS ACROSS PARTNER INSTITUTIONS AND PROGRAMS IN PUERTO RICO

Introduction

The *PR-INBRE Summer Student Research Symposium 2025*, titled "Voices of Discovery: Student Research Presentations across Partner Institutions and Programs in Puerto Rico," was held at the University of Puerto Rico Medical Sciences Campus on August 5, 2025. The symposium brought together undergraduate and graduate students, mentors, and institutional partners to present the outcomes of summer research, training, and mentorship experiences supported by the Puerto Rico IDeA Network of Biomedical Research Excellence (PR-INBRE).

This event represented a key component of PR-INBRE's workforce development strategy, providing a structured space for dissemination of student-led research, interdisciplinary exchange, and strengthening of collaborative networks across Puerto Rico and partner institutions in the mainland United States. Students presented projects spanning biomedical, behavioral, environmental, and translational sciences, reflecting both local research priorities and global scientific challenges. The symposium integrated contributions from five complementary training and mentorship programs that collectively support the development of Puerto Rico's biomedical research pipeline. These programs differ in structure and focus—ranging from community-engaged science education to advanced translational cancer research—but share a common commitment to experiential learning, mentorship, and scientific capacity building.

Program Descriptions

1. U54 Mentoring and Research Program – University of Puerto Rico Medical Sciences Campus.

The U54 Educational Program, developed through a longstanding collaboration between the University of Puerto Rico and the University of Texas MD Anderson Cancer Center, is designed to train future scientists and health professionals in cancer research through immersive, mentored experiences. Students participate in intensive summer placements of up to eight weeks, engaging in laboratory-based research under the guidance of leading investigators. During this time, they develop core competencies in experimental design, data analysis, and scientific communication. The program emphasizes a translational research approach by integrating laboratory training with exposure to clinical settings, allowing participants to observe the application of scientific discoveries in real-world healthcare contexts. Trainees receive continuous mentorship, present their findings in academic forums, and may contribute to peer-reviewed publications. In addition, the program supports long-term career development pathways, including opportunities for combined MD/PhD training, thereby strengthening Puerto Rico's capacity in oncology

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Supplement Information
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research and clinical translation. This program was supported from 2001 to 2025 through funding from the National Cancer Institute of the National Institutes of Health (NIH-NCI) under cooperative agreement grants U54 CA096297 and U54 CA096300, and was implemented at the University of Puerto Rico through a formal partnership with the University of Texas MD Anderson Cancer Center in Houston and coordinated by Dr. Ilka Ríos.

2. Semilla Program – University of Puerto Rico Medical Sciences Campus.

The *Semilla* program (R25GM137368), directed by Dr. Maribel Campos Rivera, applies an eco-bio-developmental (EBD) framework to promote emotional and physical well-being among children ages 9–12. The initiative integrates science, education, medicine, and community engagement to strengthen children's ability to understand and communicate their needs while fostering resilience and health agency. Implemented in partnership with the Boys & Girls Clubs of Puerto Rico, the program combines social-emotional learning with scientific inquiry, positioning children as active participants in their own well-being. In parallel, Semilla includes a fellowship component for educators and scientists, and supports the development of a clinical-community response to children's health needs. This model extends the concept of research training beyond traditional academic settings by embedding science within community and developmental contexts.

3. PUCPR Summer Internship Research Program – Pontifical Catholic University of Puerto Rico.

The PUCPR Summer Internship Research Program, coordinated by Dr. Zaira Mateo-Mayol, provided undergraduate students with a structured, month-long research and professional development experience during June 2025. The program supported eight STEM students and integrated laboratory training with soft-skills development, including scientific communication and career exploration. A key strength of this program lies in its collaborative framework. Students participated in PR-INBRE's Undergraduate Summer Internship Kick-Off Week, gaining exposure to research conducted at the Molecular Sciences Research Center, and benefited from a partnership with Vanderbilt University, where selected participants engaged in specialized laboratory training. Additionally, the program contributed to broader recruitment and pipeline development through participation in the PR-INBRE Educational Tour, connecting students to graduate training opportunities across institutions.

4. Summer Undergraduate Research Experience (SURE) – Universidad Ana G. Méndez, Carolina Campus.

Directed by Dr. Loyda B. Méndez, this summer experience provides undergraduate students with research training in Biomedical Sciences through funds from the US Department of Education (PR Award #P031S230161). Participants gain exposure to laboratory techniques, responsible conduct of research and science communication. At the conclusion of the summer, students present their results at local and national symposiums.

Funding Statement
This supplement and the research presented were supported by the Puerto Rico IDeA Network of Biomedical Research Excellence (PR-INBRE), funded by the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health under Award Number P20GM103475, and by related training and research grants supporting participating programs.

Special Thanks
Special thanks to Ms. Sandra Charriez, Assistant Project Coordinator of the Science and Technology Competency Education (STCE) Core, Puerto Rico IDeA Network Biomedical Research Excellence, for her support for the success of this summer activity.

We want to congratulate all the students and mentors presenting your amazing work. Your dedication, curiosity, and resilience are the true engine of scientific progress, and it has been a privilege to witness your growth, creativity, and commitment to advancing knowledge that will impact Puerto Rico and beyond.

"As I close my 25-year journey with this program, I am deeply grateful for the opportunity to share my passion for research with all of you. To our students: you are the future of science, innovation, and leadership. Carry forward this spirit of inquiry, support one another, and never lose sight of why you started. The path is challenging, but the impact is profound. Science is not just what we discover, it is what we dare to imagine and the lives we choose to change."
By Dr. José F. Rodríguez Orengo

Keywords
PR-INBRE; Undergraduate Research; Biomedical Sciences; Research Training; Puerto Rico; Translational Research; Student Symposium

5. University of Wisconsin Graduate School's Partnership Program in Graduate Excellence (PPGE) with Universidad Ana G. Mendez, Carolina Campus.

Co-led by Dr. Loyda B. Méndez at UAGM-Carolina and Dr. Ari Rosenberg at University of Wisconsin, this partnership with the UW Neuroscience Training Program (UW-NTP) promotes scientific collaboration and research opportunities in resource-limited primarily undergraduate institutions. The UW-NTP collaborates with faculty and students at UAGM-Carolina to develop science outreach workshops, create opportunities for scholarly visits for faculty and students, recruit postbaccalaureate and graduate students to UW-Madison, and facilitate research collaborations between the universities.

6. West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) - Marshall University and West Virginia University.

The STCE organized a collaboration between the Puerto Rico IDeA Network of Biomedical Research Excellence (PR-INBRE), supported by NIH/NIGMS under Grant No. P20GM103475, and the West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) supported by NIH-NIGMS Grant No. P20GM103434. The NIGMS IDeA Networks of Biomedical Research Excellence (INBRE) supports statewide biomedical research and training programs supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH). PR-INBRE and WV-INBRE participate in a joint summer student research exchange, expanding research training opportunities beyond their respective home institutions. Through this partnership, students from Puerto Rico and West Virginia have the opportunity to participate in summer research internships at each partner institution, work alongside faculty mentors, gain exposure to different and novel research environments and scientific approaches, and present research and engage in professional development activities. A key goal of this exchange program is to establish lasting connections among students from Puerto Rico and West Virginia that continue beyond the summer experience. Upon returning to their home institutions for the regular academic year, students and their respective mentors are encouraged to maintain communication and explore opportunities for continued collaboration, including sharing research experiences, scientific interests, and potential academic and research initiatives. This sustained interaction strengthens the connection between our INBRE programs, promotes the exchange of knowledge and expertise, and fosters a broader, more collaborative biomedical research community.

7. Science, Technology, and Career Enhancement (STCE) Component – Puerto Rico INBRE.

The STCE component serves as the central training and coordination platform within PR-INBRE, supporting students across participating institutions through mentorship, research placements, and career development activities (Award P20GM103475). STCE facilitates integration across programs by organizing workshops, symposia, and networking opportunities that enhance scientific skills and professional readiness. Through its cross-institutional structure, STCE promotes continuity in training experiences, supports student progression along the biomedical pipeline, and fosters a collaborative research culture. Its role in coordinating initiatives such as the Summer Student Research Symposium underscores its importance in advancing equity, inclusion, and sustained workforce development in Puerto Rico's scientific ecosystem. The STCE team is composed of a multidisciplinary group of investigators, educators, and program coordinators who contribute to the design, implementation, and evaluation of training activities across the network. Core team members include Drs. Loyda Méndez, José F. Rodríguez-Orengo, Juan López-Garriga, and Edna Acosta Pérez.

Overall, the 2025 symposium served as a critical platform for students and mentors to network, share experiences, and present the outcomes of their summer internships and training activities. The integration of these five programs highlights a coordinated, multi-level approach to research training that spans community engagement, undergraduate education, and advanced translational research, reinforcing Puerto Rico's commitment to building a sustainable and collaborative biomedical research infrastructure.

Theme 1: Neuroscience and Behavioral Sciences

This theme explored neural mechanisms, cognitive behavior, and neurotoxicity through a range of experimental models, including *Drosophila melanogaster*, *Caenorhabditis elegans*, and mammalian systems. Projects examined molecular mechanisms underlying epilepsy, neuroplasticity, and Parkinson's disease, as well as the effects of stress and microgravity on brain function. Students applied innovative assays to investigate behavioral regulation and neural adaptation, advancing understanding of neurological resilience and vulnerability in disease contexts.

Modulating Macrophage Interactions with the Blood Brain Barrier: Edelfosine as a Potential Therapeutic in Epilepsy-Associated Inflammation

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Seizures and epilepsy disrupt the blood-brain barrier (BBB), allowing macrophages to enter the brain and release pro-inflammatory cytokines that worsen neuronal hyperexcitability. This study explores edelfosine, an alkyl-lysophospholipid, as an anti-inflammatory agent in seizure-like conditions. Previous work showed that edelfosine reduces cytokine transcription in microglia. Here, a macrophage adhesion assay was performed using murine brain endothelial cells (bEnd.3) treated with pilocarpine. Fluorescently labeled macrophages were added to assess whether edelfosine limits their adhesion to inflamed BBB cells. A macrophage adhesion assay was performed using bEnd.3 endothelial and RAW 264.7 macrophage cells. Cells were cultured in DMEM with 10% FBS, treated with various compounds, and incubated for up to 48 hours. Fluorescently labeled macrophages were added, fixed after adhesion, and analyzed via confocal microscopy using ImageJ software. Pilocarpine (10 µM) significantly increased macrophage adhesion compared to vehicle, confirming its pro-inflammatory effect on the BBB endothelial (bEnd.3) cells. However, treatment with edelfosine (1 µM)—with or without pilocarpine—reduced macrophage adhesion, though not as effective as dexamethasone (50 µM; positive control) which also decreased macrophage adhesion when combined with pilocarpine. The results support the hypothesis that edelfosine modulates immune-endothelial interactions, probably by reducing proinflammatory mediators involved in macrophage recruitment and regulating the expression of adhesion proteins in endothelial cells. Preliminary data indicate that edelfosine significantly decreases macrophage adhesion to BBB endothelial cells, comparable to the effect observed with the corticosteroid dexamethasone. These findings suggest that edelfosine may exert its anti-inflammatory effects, in part, by limiting macrophage recruitment to the CNS following seizure-like insults. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Drosophila melanogaster as a Research Model to Study the Impact of PFAS on Neuroplasticity

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants known for their stability and bio accumulative properties, raising concerns about their potential to disrupt biological systems. This study used Drosophila melanogaster to evaluate the effects of PFAS exposure on survival, reproduction, locomotor behavior, and ethanol tolerance—traits associated with neuroplasticity. For the survival analysis, male and female flies were exposed to PFAS concentrations ranging from 1 µM to 1 mM for 28 days. Reproductive effects were assessed by allowing 10 mating pairs to reproduce for 3 days under 100 µM, 300 µM, 700 µM, and 1 mM PFAS, and offspring were counted over 7 days. Locomotor performance was evaluated using the Rapid Iterative Negative Geotaxis (RING) assay after 14 days of exposure to 1µM-to-1mM concentration range. To assess alcohol tolerance, flies were exposed to 300 µM, 700 µM, and 1 mM PFAS for 14 days, followed by an acute ethanol tolerance assay. Our findings indicate that PFAS impacts survival in a concentration- and sex-dependent manner. Reproductive output decreased significantly with increasing concentrations. Locomotor ability was affected in a dose-dependent manner, with 300 µM producing the most significant reduction, though no sex differences were observed. Ethanol tolerance development shows a significant difference in time to reach 50% sedation time by PFAS exposure. These results suggest that PFAS may impair survival, reproduction, and motor behavior in *Drosophila* in a concentration-dependent fashion. Further research is needed to confirm these preliminary findings and to understand the mechanisms involved in PFAS-induced neurotoxicity. **Acknowledgements**: I would like to acknowledge: Eowyn García, Amanda Gonzalez, Kiarelys Castrodad, Alex Rodriguez, Zara Blasini, Nayelis Rosario, Priscila Gutierrez. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Testing the Necessity of LB23 Neurons in Drosophila Grooming Behavior

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Abnormally repetitive behaviors are hallmark symptoms of disorders like autism spectrum disorder and obsessive-compulsive disorder. These behaviors have been linked to disruptions in brain regions that select and execute movement sequences. Therefore, elucidating the neural pathways involved in sequential actions is crucial for understanding these disorders. We study grooming behavior in *Drosophila melanogaster*—a predictable sequence of leg movements—as a model for neural organization of sequential actions. Our previous work with the FlyWire 3D electron microscopy dataset identified a hemilineage of brain neurons (LB23) that shows high connectivity and receives input from bristle mechanosensory neurons. We also found that optogenetic activation of LB23 subsets via CsChrimson elicits grooming of specific head locations. Additionally, simultaneous inhibition of most LB23s causes severe head grooming defects. We hypothesize that LB23 neurons are not only sufficient but also necessary for proper head grooming. To test this, we expressed hid in specific LB23 subsets using five split-GAL4 lines—the same used for activation—to induce cell death, along with a split-GAL4 control. We also used R77C10-GAL4 for broad LB23 inhibition with a corresponding control. Flies coated with dust groomed for 1.5 minutes; impaired grooming resulted in dust accumulation on specific head regions. Inhibition of LB23 subsets via ant-LB23-spGAL4 and eye-LB23-spGAL4 lines caused mild dust buildup, suggesting inhibition of these subsets may cause slight impairment, but they are not

essential. Inhibition via nod-LB23-spGAL4 produced consistent and moderate dust accumulation, indicating a critical role in grooming. As expected, broad LB23 inhibition via R77C10-GAL4 resulted in the most severe grooming defects. **Acknowledgements**: Seeds & Hampel Lab, Murthy and Seung Labs, Jonathan Blagburn, & Jacob Austin Johnson. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Expression of the dct-1 Gene in the sod-1(G85R)c Strain in *Caenorhabditis elegans*

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the death of motor neurons. ALS affects the control of voluntary movements in arms, legs, and eventually the breathing. The SOD1 gene was the first gene related to ALS. The SOD1 gene express an antioxidant enzyme which protects the cell from the toxicity of reactive oxygen species. This gene is conserved in the Eukarya Domain. One of the hundred of mutation in SOD1 gene associated to ALS is G85R, a glycine substitution to arginine residue positioned 85 in the SOD1 protein. This study used homologous mutations in endogenous sod-1 gene of the nematode, C. elegans. The sod-1(G85R)c strain display locomotion defects, neurodegeneration of motor neurons and mitophagy impairments. We will evaluate the expression of the gene dct-1 in sod-1(G85R)c strain. The dct-1 gene is receptor protein located into the mitochondrial outer membrane, related to apoptosis/cell-cycle and autophagy/mitophagy. The dct-1 gene is an ortholog of human BNIP3 gene. Mitophagy is a type of autophagy specifically to remove damaged or aged mitochondria. The mitophagy pathway is a highly regulated process that involves the stepwise recruitment of multiple proteins. Mitophagy is considered as a factor in the homeostatic pathways in neurons. We propose that the expression of gene dct-1 is affected in sod-1(G85R)c strain. In order to determine gene expression, we measured the mRNA levels of dct-1 gene using Real Time PCR. The gene expression analysis conveyed that the dct-1 gene in the strain sod-1(G85R)c is upregulated due to a fold change of 2. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Neurological Effects of Simulated Microgravity in a *Caenorhabditis elegans* Parkinson's Disease Model

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the accumulation of alpha-synuclein aggregates that affect dopaminergic neurons. Prolonged space missions expose organisms to microgravity, a condition that increases oxidative stress and disrupts dopamine systems, mimicking aspects of PD. In this study, the NL5901 strain of *Caenorhabditis elegans* at l4+2 larvae stage, which expresses alpha-synuclein-YFP in body wall muscles, was used to evaluate the effect of simulated microgravity on motor behavior using the Nose Touch Avoidance assay and on alpha-synuclein aggregation through fluorescence analysis. Welch's T-tests were applied to compare control and experimental groups. No statistically significant differences were observed in motor behavior or in alpha-synuclein fluorescence between groups. Simulated microgravity conditions did not induce

significant changes in the analyzed parameters. These preliminary results highlight the need to optimize the protocol and increase sample size for future studies, including the evaluation of angiotensin-converting enzyme inhibitors such as captopril in PD models. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Validation of Proteins Associated with Anxiety-like-behavior in *Drosophila melanogaster* through Exposure to *Stypopodium zonale* Extracts

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Proteins play a critical role in cellular functions, mediating biochemical pathways that regulate stress responses, neuronal activity, and behavior. Natural compounds from marine algae, known for their bioactive metabolites, hold promise for modulating these processes. This study investigates the effects of organic extracts from *Stypopodium zonale* on proteomics changes and behavior using *Drosophila melanogaster* as a model organism. We hypothesize that the proteins related to anxiety-like-behavior are going to be less expressed in the flies that are exposed to the extract. To conduct this, protein extraction of *Drosophila's* heads was carried out, followed by protein quantification to determine their concentration. To further understand the molecular mechanisms, quantitative proteomics by mass spectrometry and bioinformatics analysis was performed at the *Proteomics Center*. Results revealed fifty-three differentially abundant proteins, eleven of those were found to be associated with anxiety-like- behavior and five significant proteins were selected for validation. To validate these changes in protein expression between the control and experimental group we used Western Blotting techniques. A loading control antibody was used to confirm equal protein concentration in each lane; band densitometry analysis revealed variable expression on *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)*. Upon arrival, an antibody against *Synaptosomal-associated protein 47 (SNAP-47)* will be validated using *GAPDH* or actin as loading controls. This multi-step proteomics approach aims to elucidate how marine algae-derived compounds affect Drosophila's head proteome. By identifying proteins and signaling pathways that are altered by these compounds, the study contributes to the understanding of their potential as alternative pharmacotherapies for the treatment of stress-related disorders. **Funding**: This research was supported by an Institutional Developmental Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Effects of Captopril in Locomotion and Fluorescence Assay in *Caenorhabditis elegans* Parkinson's Disease Model

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor impairment, primarily caused by the accumulation of alpha-synuclein leading to neuronal degeneration. Captopril, an angiotensin-converting enzyme inhibitor used for hypertension, has shown neuroprotective effects by reducing oxidative stress, neuronal loss, and neuroinflammation. This study evaluated the effects of captopril on the avoidance response to Nose Touch Avoidance (NTA) and fluorescence intensity in the *Caenorhabditis elegans* (*C. Elegans*) at L4+2 days larvae

stage, PD model NL5901, which expresses human alpha-synuclein. Synchronization and treatment assays were performed, followed by behavioral (NTA) and fluorescence analyses after two days of exposure. Results showed no significant differences between control and experimental groups in either NTA or fluorescence intensity. These findings contrast with previous assays conducted after longer exposure (L4 + 7 days), which demonstrated significant improvements in motor behavior, suggesting that treatment duration may influence the neuroprotective effects of captopril. Future research will explore the effects of candesartan and other ACE inhibitors, as well as replicate these experiments in the *C. elegans* PD model BZ555 to further elucidate potential therapeutic benefits.
Funding: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Influence of Alcohol and Western Diet on Cerebral Perfusion and Gut Microbiome Diversity in Alzheimer's Disease Risk

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Alzheimer's disease (AD), a type of dementia, is characterized by amyloid-beta (Aβ) accumulation, tau pathology, progressive cognitive decline and often comorbid cerebrovascular disease. Modifiable lifestyle factors, particularly diet and alcohol consumption, have been implicated in dementia risk through their effects on cerebrovascular integrity and cerebral perfusion. Gut microbiome diversity influences brain health and may affect cerebral blood flow (CBF) through gut-brain axis pathways. Multi-post labeling delay arterial spin labeling (MD ASL) MRI provides a noninvasive measure of cerebral blood flow (CBF) corrected for arterial arrival time, enabling early detection of neurovascular dysfunction. This study examined associations between modifiable lifestyle factors including western dietary patterns and alcohol intake in cortical CBF and gut microbiome diversity from MD ASL in cohorts at risk for dementia due to AD. Data were collected from middle- aged to older adults at increased risk for AD (full cohort: n=122, mean age = 68 years), of whom 68% were female. One-third (34%) were APOE ε4 carriers, a known genetic risk factor for Alzheimer's disease. Participants were drawn from the Wisconsin Registry for Alzheimer's Prevention (WRAP) and the Wisconsin Alzheimer's Disease Research Center (ADRC). Western diet scores were derived via principal component analysis of food frequency questionnaires (FFQ); alcohol intake was quantified as average daily units; cortical CBF was derived from MD ASL MRI scans. Adjusted linear regression models revealed a positive association between alcohol intake and gray matter CBF (** $P < 0.014$) in the frontal and temporal lobes, primarily driven by high- intake outliers (>5 drink/day), while Western diet showed no significant effect. Women showed higher CBF compared to men. Vascular and metabolic covariates did not have a significant effect on perfusion. In a secondary aim, gut microbiome diversity metrics were analyzed in relation to CBF. Faith's Phylogenetic Diversity (PD), an alpha diversity metric reflecting within-individual evolutionary microbial richness, was positively associated with CBF. Beta diversity analyses using Bray-Curtis dissimilarity index revealed significant sex-dependent differences in gut microbial community; however, this metric was not associated with CBF. While Western diet was not associated with perfusion, we observed that phylogenetic richness of gut microbiota may influence cerebrovascular function and brain blood flow. The results of the alcohol consumption analysis were limited given the few participants had high intake but suggested that alcohol may also impact cerebral perfusion. These results point toward potential pathways through which modifiable factors could impact dementia risk via cerebrovascular mechanisms.

Theme 2: Cancer Biology and Therapeutics

Research in this area addressed mechanisms of tumor development, progression, and drug resistance across multiple cancer types. Students investigated the cytotoxic effects of natural products, drug synergies in chemotherapy, and molecular targets such as TRIP13, Zeb1, and FASN. Studies integrated biochemistry, pharmacology, and proteomics to elucidate therapeutic mechanisms and resistance pathways. Collectively, these works reflected a growing emphasis on translational oncology and the development of precision interventions for aggressive or treatment-resistant cancers.

Cytotoxic Effect of Ergosterol Peroxide in Triple Negative Breast Cancer Model

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Triple-negative breast cancer (TNBC) is a particularly aggressive subtype of breast cancer, accounting for 15% to 20% of all breast cancer diagnoses. It's characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. For patients with metastatic TNBC, the median survival is less than 18 months. Ergosterol peroxide (EP) is one of many bioactive compounds from *Ganoderma lucidum*, a medicinal mushroom known for its anti-cancer properties. Previous studies show, EP and its derivatives do not cause cytotoxic effects in normal human fibroblasts (BJ cell lines), epithelial cells (MCF10A), or cardiomyocytes, suggesting a favorable safety profile. In contrast, it has demonstrated potent, concentration-dependent anti-cancer activity in TNBC cell lines, specifically, MDA-MB-231. This selective effect on tumor cell viability suggests a promising therapeutic window. In this work, we propose to conduct washout assays to analyze the cancer cells after EP withdrawal and determine what changes occur in their viability. MDA-MB-231 cells (15,000/well) were seeded in a 48-well plate. After 24h, media was changed to 5% FBS for 1h. Cells were treated with EP, EP-2, EP-3 for 72h, then the media was changed for an additional 72h. Cell viability was analyzed by PI, and graphed. Results show reduced IC50s post washout indicating the compounds exert long-term cytotoxicity.

Evaluation of Ergosterol Peroxide in the Modulation of Inflammation in Lipopolysaccharide-Activated Macrophages

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Triple-negative breast cancer (TNBC) is a highly aggressive subtype with limited therapeutic options. Among the immune cells within the tumor microenvironment, tumor-associated macrophages (TAMs) are found in high numbers and display functional activities. When TAMs are stimulated by pro-inflammatory signals such as lipopolysaccharide (LPS), they adopt a pro-tumoral phenotype that promotes cancer progression. Our studies show that Ergosterol Peroxide (EP), a medicinal mushroom-derived compound, displays cytotoxic activity against TNBC cells. However, the immunomodulatory effects that EP may have on macrophages remain poorly understood. Therefore, this study investigates whether EP and its derivatives, suppress LPS-induced macrophage activation and whether this suppression influences macrophage cell viability. RAW 264.7 macrophages were cultured and treated with increasing concentrations of EP or derivatives (0–50 μM) under three protocols:

EP exposure alone, LPS preactivation (2h) followed by EP, or EP treatment followed by a short (30 min) LPS stimulation. Cell viability was assessed using methanol fixation, propidium iodide staining, and fluorescence detection with a GloMax reader. The results demonstrate a progressive decrease in the IC_{50} value of EP under inflammatory conditions. Under basal conditions, the EP, EP-2 and EP-3 IC_{50} were 5.7, 4.0, 8.4 μM, respectively. When cells were pretreated with LPS for 2 hours before EP exposure, the IC_{50} changed to 14, 5.2, 2.7 μM, respectively. Remarkably, when LPS was added 30 minutes after a 72-hour EP treatment, the IC_{50} changed to 6.5, 4.2, 2.3 μM. These findings suggest that an inflammatory environment enhances macrophage sensitivity to the EP-3 derivative, possibly through LPS-induced alterations in signaling pathways, oxidative stress, or cellular metabolism. Changes in parental EP were not as responsive, while derivative EP-2 did not display changes among the conditions. In conclusion, derivative EP-3 exhibits increased cytotoxic efficacy in RAW 264.7 cells under LPS-induced inflammatory conditions, indicating that pro-inflammatory environments may positively modulate its activity. This reinforces the therapeutic potential of EP in inflammatory or immune-mediated diseases, including certain types of cancer such as TNBC. **Funding**: This research was supported by an Institutional Development Award (IDea) National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health under grant number P20GM103475, NIGMS grant number R16GM145488 to M.M.M.; and Louisiana Board of Regents Support Fund Award LEQSF-RD-A-05 and NIGMS grant number R15GM148983 to F.R and NIGMS under award numbers U54GM133807.

Antitumor Activity of Diosgenin Combined with Chemotherapy in KRAS-mutant and EGFR-mutant Non-Small Cell Lung Carcinoma Cells

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No small cell lung carcinoma (NSCLC) accounts for 85% of lung cancers and remains the leading cause of cancer mortality. Standard chemotherapies such as cisplatin and doxorubicin suffer from acquired resistance, metastasis, and toxicities, particularly in KRAS mutant (~28 %) or EGFRmutant (~15 %) tumors. Consequently, innovative adjuvant strategies are needed. Plant derived compounds are promising sources of anticancer agents. Diosgenin, a steroidal sapogenin, has demonstrated antitumor properties. Here, we investigated the potential of diosgenin to enhance the efficacy of cisplatin and doxorubicin in KRAS mutant (A549) and EGFRmutant (H1975) NSCLC cell lines. A549 and H1975 cells were treated for 24 hours with diosgenin, cisplatin, and doxorubicin as single agents and in dual drug combinations (diosgenin with cisplatin or doxorubicin). Cell viability was measured after treatments by MTS assay, and IC_{50} values were determined by nonlinear regression. Synergistic, additive, or antagonistic drug interactions were assessed using Synergy Finder 4.0. As a single agent, diosgenin induced dose-dependent cytotoxicity in both NSCLC lines with IC_{50} values in the micromolar range and strongest potency in H1975 cells. The effects of combination therapy were highly dependent on dose and cell type. In A549 cells, diosgenin combined with either cisplatin or doxorubicin showed synergy at mid-range concentrations but antagonism at high doses. While the combinations were overall antagonistic in H1975 cells, a similar window of mid-dose synergy was still observed, particularly with cisplatin, highlighting a complex interaction profile. These findings support the further preclinical investigation of diosgenin and emphasizes need for supervised precise dose optimization in dual drug combinations. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Plant Nanosuspensions Interacting with Chemotherapy and Rewiring Cancer Metabolism in NSCLC

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Non-small cell lung carcinoma (NSCLC) remains a leading cause of cancer mortality, with chemoresistance limiting the efficacy of standard drugs like cisplatin and doxorubicin. Many patients concurrently use over-the-counter plant supplements, often under the misconception of universal safety. This study investigates how nanosuspensions (NS) from oregano, graviola, and cinnamon interact with chemotherapy and affect key signaling pathways in KRAS-mutant (A549) and EGFR-mutant (H1975) NSCLC cells. Stable aqueous phyto-NS were prepared and characterized. To evaluate drug interactions, A549 and H1975 cells were co-incubated with the phyto-NS and either cisplatin or doxorubicin. Cell viability was assessed by MTS assay and synergy was quantified using the Synergy Finder platform. Effects on cellular metabolism were assessed via flow cytometry, by DNA damage machinery assay. Phyto-NS alone induced dose-dependent cytotoxicity and an increase DNA double strand breaks and histone phosphorylation. In combination studies, only oregano NS demonstrated synergistic and additive effects at low-mid concentrations with cisplatin in A549 cells, enhancing its therapeutic potential. Conversely, these phyto-NS exhibited significant antagonism when combined with doxorubicin in A549 cells. In H1975, all phyto-NS exhibited antagonism when combined with cisplatin and doxorubicin. Plant-based supplements can have profound and unpredictable interactions with conventional chemotherapy. While some combinations may be beneficial, such as the observed synergy/additive with cisplatin KRAS-mutant A549, others can be detrimental. These findings challenge the "natural is safe" paradigm, highlighting the critical need to evaluate supplement-drug interactions to guide safer and more effective integrative oncology strategies. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Effect of Nicotine on the Growth of Human Ovarian Clear Cell Carcinoma

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Human ovarian clear cell carcinoma (OCCC) is a rare and aggressive subtype of epithelial ovarian cancer that often presents at advanced stages, where it exhibits high resistance to conventional chemotherapy and is associated with poor prognosis. Interestingly, epidemiological studies have suggested that smoking may reduce the risk of developing OCCC. Nicotine is the addictive component of cigarette smoke. Published data show that nicotine regulates the growth of human tumors via the nicotinic acetylcholine receptors (nAChRs) on target cells. The central hypothesis of our study is that nicotine inhibits the growth and progression of human OCCC. The first series of experiments examined the effect of nicotine on the viability of human OCCC cell lines (TOV21G and SKOV3), using the MTT assay. We observed that nicotine inhibited the viability of TOV21G and SKOV3 cells in a concentration-dependent manner over a period of 24 hours. The concentration of nicotine in the blood of moderate smokers ~100nM-10μM. MTT assays showed that nicotine (within the range of concentrations found in smokers) robustly decreased the viability of TOV21G and SKOV3 cells. Our future experiments will delineate the signaling pathways responsible for the growth-suppressive activity of nicotine in human OCCC cells. Such research would facilitate the identification novel molecular targets relevant for the therapy and management of OCCC. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475 (PRINBRE)and P20GM103434 (WVINBRE).

Region B Capsaicin Analogs Display Robust Growth-Suppressive Activity in Human Ovarian Cancer Cells

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Ovarian cancer (OC) is the most lethal gynecological malignancy in women, with a five-year survival rate of only ~50%. The standard first-line treatment involves cytoreductive surgery followed by chemotherapy with platinum-based drugs such as cisplatin. However, the development of chemoresistance remains a major clinical challenge. These concerns highlight the urgent need for novel therapeutic approaches. The nutritional compound Capsaicin, the spicy compound in chili peppers, has been shown to exert growth-inhibitory activity in a diverse array of human cancers, including human OCs. Nonetheless, its clinical application is limited due to adverse side effects. One strategy to overcome this is the design of non-pungent capsaicin analogs that retain anticancer activity. Capsaicin's pharmacophore includes three structural regions (A, B, and C). In this study, we examined three analogs with modifications in Region B: capsaicin, capsiate, and thioamino-capsaicin (T-CAP). We screened their growth-suppressive activity using an MTT-based assay in OVCAR-3 and SKOV3 OC cell lines. Cells were treated for 24 hours, and viability was measured via absorbance after MTT reduction and formazan solubilization. Among the analogs, T-CAP produced the greatest decrease in OC cell viability. Importantly, these analogs did not affect normal lung epithelial cell viability, suggesting selective toxicity. Future studies will explore the molecular mechanisms responsible for T-CAP's effects, including potential TRPV1 receptor involvement. These findings support the potential of nutrition-based analogs as safe and effective therapeutic candidates for OC.
Funding: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475(PRINBRE) and P20GM103434 (WVINBRE).

Proteomic Profiling of Therapy-Resistant Prostate Cancer Cells

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Prostate Cancer (PCa) affects millions of men worldwide and is the fifth leading cause of cancer- related death in men. The prostate (a key organ in the male reproductive system) is strongly regulated by androgens, making this cancer highly dependent on hormones and its environment for growth. Docetaxel-based chemotherapy remains a standard treatment for advanced PCa; however, it is not curative, and patients often progress to lethal chemotherapy resistant disease. To investigate the mechanisms of chemoresistance, we used docetaxel-sensitive and -resistant prostate cancer cell lines. This study analyzed proteomic data generated using LC-MS/MS to identify central mechanisms of docetaxel resistance in prostate cancer. Data from these analyses were further examined using Principal Component Analysis (PCA), volcano plots, and proteomic databases (String and Met), to identify biological pathways involved in docetaxel-sensitive and - resistant cell lines. Protein lysates from 22Rv1, PC3, and DU145 prostate cancer cell lines and their docetaxel-resistant sublines were collected. Immunoblotting techniques were then employed to validate proteomic findings in cell-based models of chemotherapy resistant prostate cancer. Our analyses revealed that docetaxel-resistant prostate cancer cells exhibit distinct intracellular signaling compared to their parental counterparts. Notably, in the PC3/PC3DR comparison, the c-Jun signaling pathway emerged as a key mechanism given its role in cell survival, growth, and metabolic reprogramming,

representing a therapeutic vulnerability. Future work will expand proteomic analyses to the 22Rv1, DU145, and their resistant sublines models and explore strategic targeting of resistance-associated pathways to evaluate metabolic vulnerabilities in chemotherapy resistant prostate cancer. **Funding**: This research was supported by an Institutional Developmental Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Characterization of Extracellular Vesicles from Therapy Resistant Prostate Cancer Cells

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Prostate cancer (PCa) remains one of the most prevalent malignancies affecting men worldwide. Docetaxel is a chemotherapeutic agent for treating metastatic castration-resistant prostate cancer (mCRPC); however, the emergence of drug resistance poses a significant challenge to effective therapy. Investigating docetaxel-sensitive and -resistant prostate cancer cell lines provide insight into the molecular mechanisms driving resistance and which may help in the development of improved treatment strategies. Exosomes are small extracellular vesicles (30–150 nm) that mediate intercellular communication and play important roles in tumor progression, immune regulation, and therapy resistance; primarily through the transfer of proteins, lipids, and nucleic acids. In particular, tumor-derived exosomes (TDEs) influence how cancer cells interact with the immune system and contribute to tumor dynamics. Understanding these mechanisms requires robust and efficient exosome isolation and characterization methods. To this end, we used qEV isolation columns for size exclusion chromatography (SEC) to isolate and purify extracellular vesicles from DU145, 22Rv1, and PC3 prostate cancer cell lines both docetaxel-sensitive and - resistant variants. Extracellular vesicles were successfully purified and identified, and the results were validated via Western blotting using established positive and negative extracellular vesicle markers. Western blotting of cell lysates was also performed to assess sample contamination and confirm the reliability of the isolation. Dynamic light scattering analysis confirmed particle sizes within the expected for extracellular vesicles with sizes between 100 nm and 230 nm, consistent with extracellular vesicle profiles. Together, these validation techniques demonstrated the efficiency and specificity of our protocol for exosome isolation and characterization. This optimized methodology represents a critical first step toward exploring the role of exosomes in tumor behavior and understanding their contribution to cancer progression and drug resistance. **Funding**: This research was supported by an Institutional Developmental Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Identifying Novel Zeb1 Targets in Murine Cell Lines of Non-Small Cell Lung Cancer

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths among both men and women in the United States and worldwide, largely due to poor prognosis when diagnosed at the metastatic stage. Metastatic cancer cells frequently undergo Epithelial-to-Mesenchymal Transition (EMT) in order to migrate away from the main tumor mass. A key regulator of EMT is the double-negative feedback loop between the microRNA-200 family and

the transcription factor Zinc finger E-box-binding homeobox 1 (Zeb1). Zeb1 can induce EMT by repressing epithelial genes and pathways, or by promoting mesenchymal ones. However, not all Zeb1 targets have been identified. In this study, we analyzed candidate target genes previously identified through multi-omics sequencing comparing gene expression in the presence and absence of Zeb1. To refine the list of dysregulated genes, we applied three approaches: greater fold-change, statistical significance and functional relevance based on Gene Ontology (GO) terms using DAVID pathway analysis. We utilized cell lines derived from primary and metastatic tumors found in four different genetically engineered mouse models (GEMMs) of NSCLC, which incorporate mutation or loss of two of the most common genes in lung cancer, Kras and p53. KrasG12D/+; p53R172HΔg/+ (KPΔg), KrasG12D/+; p53R172H/R172H (KPMUT), KrasG12D/+; miR200141/c -/- (KM), and KrasG12D/+; p53R172H/R172H; miR200141/c -/- (KPMUTM). In total a panel of 13 cell lines with varying expression of Zeb1, EMT phenotypes, and metastatic potentials were used. Gene expression was validated using real-time quantitative PCR (RT-qPCR) and protein levels were evaluated by western blotting. Three genes—Cblc, Desmoplakin (Dsp), and Lamc2—were identified as significant targets, showing strong evidence of transcriptional repression by Zeb1. These findings contribute to an understanding of the gene regulatory mechanisms underlying EMT in NSCLC. To further validate these candidates, future studies should include targeted knockdown or knockouts of Cblc, Dsp, and Lamc2 in these mouse models to assess their functional roles. Additionally, translational studies may involve examining these candidate genes in human NSCLC cell lines and exploring pharmacological agents or pathways targeting their function. **Keywords**: Lung cancer; NSCLC; EMT; Zeb1; microRNA-200; metastasis; mouse model. **Funding**: U54 Partnership for Excellence in Cancer Research.

Characterization of Bladder Cancer Cell Lines with Conditional Knockout of Fatty Acid Synthase (FASN) Gene

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Non-Muscle Invasive Bladder Cancer (NMIBC) accounts for nearly 70% of bladder cancer (BLCA) cases. Novel gene therapy with Adenoviral interferon with the ability to boost immune responses and target tumor cells has been recently approved by FDA in patients with NMIBC. Previous studies have identified FASN as a key gene that is downregulated in response to treatment suggesting the role of fatty acid metabolism in treatment response. To investigate the role of FASN in therapeutic response, we developed doxycycline-inducible FASN knockdown model in UM-UC14 cell line. We hypothesized that downregulation of FASN would impact the progression of bladder cancer and alter the cells' overall lipid metabolism, basic biological properties such as proliferation, invasion, migration, and more importantly response to IFNα gene therapy with therapy. The protein's expression was conditionally knocked down in BLCA cells, and knockdown efficiency was confirmed. Functional in vitro assays included colony formation, scratch (migration) assays, and Matrigel invasion assays using crystal violet staining. Cell viability under Ad IFNα was assessed using CellTiter-Glo. Along with IFNα treatment response, cell cycle alterations were evaluated through propidium iodide (PI) staining and flow cytometry. In UM-UC14 cells, FASN knockdown was visualized in cells by reporter gene expression (RFP). Knockdown of Fasn and its target protein Fabp4 was validated by western blotting. Colony formation assay, a measure of proliferation showed no significant difference in between FASN knockdown and FASN positive cells. Two-dimensional migration assay showed that the FASN knockdown cells were relatively slower than the FASN positive cells. Matrigel invasion assay showed marked reduction in invasion capacity after FASN knockdown. Response to IFNα resistant cell line UM-UC14 showed a decrease in MOI suggesting increased sensitivity to therapy in these cells. However, there was no significant difference in cell cycle in between FASN knockdown and control cells in cell cycle suggesting other mechanism by which change in response is induced upon FASN knockdown. We will continue to explore these mechanisms in the future. We will also investigate similar treatment response in a sensitive cell line such as RT112 upon FASN knockdown. FASN knockdown altered key biological process of migration and invasion and altered response to IFNα gene therapy. Understanding mechanisms that govern treatment responses upon FASN

knockdown would facilitate developing potential therapeutic strategies with FASN modulation in patients with NMIBC. **Keywords**: FASN, downregulation, bladder cancer, viral vectors, gene therapy. **Funding**: U54 Partnership for Excellence in Cancer Research.

Arhgef39: Protein Model for Studying Interactions with Ligands and Small Proteins involved in Cancer Development

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ARHGEF39 (C9orf100) is a guanine nucleotide exchange factor with DH and PH domains that activate small GTPases, regulating cytoskeletal organization, migration, and proliferation. Overexpression in non-small cell lung cancer (NSCLC) has been associated with Rac1/P38 MAPK/ATF2 signaling, promoting tumor progression and metastasis. In silico modeling and molecular docking predicted a favorable interaction between the DH domain and Rac1, supporting its role in GTPase activation. Clinically, elevated ARHGEF39 correlates with larger tumors, increased metastatic potential, and reduced patient survival. These results suggest ARHGEF39 as a promising diagnostic biomarker and therapeutic target in NSCLC. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Evaluation of the Biological Activity of Novel BQS Compounds in Human Melanoma Cells under Normoxic and Hypoxic Conditions

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The incidence of melanoma continues to rise in the USA and worldwide. In 2025, the USA estimates approximately 104,960 new cases of invasive melanoma, with 8,430 deaths associated. A common feature of skin cancer is a low intratumor oxygen level known as hypoxia. Hypoxic cancerous skin cells are hard to treat presenting strong resistance to chemotherapy and radiotherapy. Hypoxia can also affect skin healing after skin cancer excision given insufficient oxygen supply. This study evaluates the cell toxicity of two novel 3-nitrobenzazolo[3,2-a] quinolinium chloride (BQS) compounds as well as their potential as markers of hypoxic cells in skin cancer tissues. Melanoma cells (SK-MEL-5) were exposed to ABQ-48 and NBQ-234-TOM as experimental compounds, with Cisplatin serving as the positive control and vehicle nano-pure deionized water as the negative control. Preliminary results under normoxic conditions revealed that ABQ-48 exhibited an GI_{50} of 10.75 µM, NBQ-234-TOM showed an GI_{50} of 25.92 µM, and Cisplatin displayed an GI_{50} of 40.0 µM. Cells exposed at hypoxic environment demonstrated however resistance in comparison to normoxic environment, presenting ABQ-48 a GI50 of 40.79 µM and NBQ-234TOM a GI50 of 80.3 µM under hypoxia. Cell death processes were also evaluated, including apoptosis induction (via annexin V), Caspase 3/7 activation, mitochondrial membrane permeability, and DNA fragmentation, as well as cell migration (wound healing) and colony formation assays. Results showed cell death induction by Annexin V assay, Caspase 3/7 indicated that intrinsic pathways are activated. Mitochondrial permeabilization showed more activity by NBQ-234TOM than with ABQ-48. DNA Fragmentation was also induced by BQs compounds. Skin cancer cells treated with both BQs also demonstrated a significant inhibition on migratory capacity and colony formation in contrast to controls. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Theme 3: Microbiology, Environmental, and Natural Product Research

This group of studies emphasized Puerto Rico's rich biodiversity and its potential for biomedical discovery. Students explored antimicrobial metabolites from amphibian and reptile microbiomes, air pollutant effects on liver health, and the structural characterization of plant-derived compounds. These projects showcased the island's unique ecosystem as a source of novel bioactive molecules and highlighted the intersection between environmental health and therapeutic innovation.

Functional Bioprospecting of Antimicrobial Metabolites from the Skin Microbiome of *Eleutherodactylus spp.*

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Antimicrobial resistance (AMR) is an escalating global health crisis often referred to as the "silent pandemic," fueled by the emergence of multidrug-resistant pathogens. These superbugs undermine the efficacy of conventional antibiotics, turning once treatable infections into major clinical challenges. Without timely action, AMR is projected to cause up to 10 million deaths annually by 2050, with economic losses exceeding $1 trillion. In this exploratory study, we performed functional bioprospecting of skin-associated microbial communities from *Eleutherodactylus spp.*, endemic amphibians of Puerto Rico. Cultivable microorganisms were isolated and screened for the production of bioactive secondary metabolites. Crude extracts were purified using Fast Protein Liquid Chromatography (FPLC) with Sephadex resin, and resulting fractions were analyzed via Quadrupole Time-of-Flight Mass Spectrometry (QToF MS) and molecular networking. Antimicrobial activity of purified fractions was evaluated through minimum inhibitory concentration (MIC) assays against Staphylococcus aureus. Several fractions exhibited dose-dependent inhibition, indicating the presence of potent antimicrobial compounds. No significant activity was observed against Escherichia coli, suggesting species-specific bioactivity. Preliminary metabolomic analysis revealed small molecules and cyclopeptides with diverse structural features likely responsible for the observed effects. This study is the first to investigate amphibian-associated microbiota in Puerto Rico as a source of antimicrobial metabolites. The results highlight the therapeutic potential of these natural compounds and underscore the importance of amphibian skin microbiomes in the search for new antimicrobial agents. Ongoing work includes structural elucidation and functional validation of candidate molecules for biomedical application. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Evaluation of Antibiotic Activity in the Skin Mycobiota of *Chilabothrus inornatus*

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The endangered Puerto Rican Boa (*Chilabothrus inornatus*) is a native species of snake that is susceptible to fungal infections despite having a rich skin microbiota. As part of a larger effort to characterize the microbial communities associated with the Boa, our group focuses on identifying secondary metabolites with antifungal or other antimicrobial activities. Our current work focuses on a fungal isolate, *Aspergillus sp.* (135GY), from which

we have identified a family of potentially bioactive compounds. These metabolites are being evaluated for their roles in microbial competition and defense on reptilian skin. To promote the synthesis of these, we have cultured 135GY under various conditions over several weeks. Fungi were cultured using Potato Dextran Agar (PDA), Czapek's Media Agar (CMA), and Sabouraud Dextrose Agar (SDA) at room temperature and 30 ºC. Extractions were performed after each week of growth, and the resulting extracts were evaluated to determine the antibiotic activity of these components using well-diffusion, high-resolution mass spectrometry (MALDI-ToF), high-performance liquid chromatography (HPLC), and minimum inhibitory concentration (MIC) experimentation. SDA was observed to produce antibiotics just two weeks after incubation at both temperatures. Future efforts will focus on elucidating the chemical basis of microbial interactions in this system. Our work contributes to the understanding of natural antifungal strategies and may inform future conservation or therapeutic efforts. Ultimately, this study aims to highlight the importance of metabolite-mediated interactions in shaping host-associated microbiomes, particularly in tropical reptiles and amphibians, and to inspire further research in this field. **Funding**: Supported by the NIH Grant P20GM103434 to the West Virginia IdeA Network for Biomedical Research Excellence & NIH Grant P20GM103475 to the Puerto Rico IdeA Network for Biomedical Research Excellence.

Characterization Effects of Tulareydiketone Quassinoids Isolated from *Simarouba tulae*

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Quassinoids are highly oxygenated degraded triterpenes commonly found in plants of the Simaroubaceae family. These secondary metabolites, composed of 18 to 25 carbon atoms, have demonstrated potential anticancer, antimicrobial, and insecticidal properties. As part of our ongoing search for bioactive compounds from medicinal plants, we focused on the isolation and structural characterization of the novel quassinoid Tulare diketone A (TRA), extracted from *Simarouba tulae*. TR quassinoids mixture exhibited selective cytotoxic activity against the MDA-MB-231 breast cancer cell line with IC50 of 11.12 µg/mL. Fraction TR17O was purified using silica gel column chromatography with 5% methanol in chloroform. Needle-like crystals appeared in fraction 3, which was analyzed by NMR spectroscopy. The ^{1}H-NMR spectrum revealed methyl (1.2-1.5 ppm), methoxy (3.2-3.4 ppm), hydroxyl (4.5 ppm), and vinylic (5.5 ppm) proton signals. The ^{13}C-NMR showed 28 carbon signals, including oxygenated (49-55 ppm), ketal (104 ppm), and ketone (217 ppm) carbons. Updated DEPT-135 analysis confirmed the presence of eight CH_3, seven CH_2, eigth CH, and five quaternary carbons. Additionally, preliminary 2D NMR data identified three spin systems supporting the partial structure of TRA. Further studies involving complete X-ray crystallography are required to elucidate the structure and stereochemistry of TRA fully. Parallel purifications are underway/ ongoing to isolate its stereoisomer TRB. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

AI-Guided Design and Characterization of the Broad-Spectrum Antimicrobial Peptide RV1

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Antimicrobial resistance (AMR) remains a pressing global health challenge, with projections estimating up to 10 million deaths annually by 2050 due to infections that no longer respond to available treatments. Key contributors to the rise and spread of AMR include the overuse of antibiotics, increased globalization, and limitations in traditional drug development pipelines. To address this growing threat, artificial intelligence (AI) and machine learning (ML) have emerged as transformative tools to accelerate the discovery of novel antimicrobial peptides

(AMPs), which represent promising alternatives to conventional antibiotics. In this study, we designed potential cationic and hydrophilic AMPs using AntiBP3, an ML-based tool trained to identify sequence features associated with antibacterial activity. The predicted 3D structures of the peptides were modeled using PEP-FOLD3, which suggested a propensity for stable alpha-helical conformations. Structural predictions were validated experimentally via circular dichroism (CD) spectroscopy. The lead candidate, an 18-residue peptide designated RV1, was synthesized through GenScript and confirmed by mass spectrometry. Its antimicrobial efficacy was assessed through minimum inhibitory concentration (MIC) assays against six clinically and environmentally relevant pathogens: Staphylococcus aureus, Escherichia coli, Acinetobacter baylyi, Enterococcus raffinosus, Klebsiella pneumoniae, and Pseudomonas aeruginosa. RV1 exhibited both bactericidal and bacteriostatic activity in a strain-dependent manner, with notable efficacy at low concentrations (10 µM) against A. baylyi, and higher concentrations (>90 µM) required for P. aeruginosa. These findings support the antimicrobial potential of RV1 and underscore the utility of AI-assisted AMP discovery as a strategic approach to combat the AMR crisis. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Hepatic Stress in Juvenile Mice Exposed to Diesel Exhaust Particles

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Air pollution is a significant public health risk, with an estimated 7 million premature deaths annually worldwide. Diesel exhaust particles (DEP), a key component of city air pollution, are particularly dangerous due to their small size and ability to extra pulmonary organs. Studies increasingly suggest a link between exposure to air pollutants and liver damage, including conditions such as non-alcoholic fatty liver disease (NAFLD), the most relevant chronic liver illness. This project focused on identifying early liver changes after DEP exposure during a vulnerable stage of development. Liver tissues were obtained from a prior study in which juvenile mice (PND 25–33) were intranasally exposed to 0 (control), 1.0, 2.5, or 5.0 µg/dose of DEP. In this project, we processed the tissues for biochemical and spectroscopic analyses. Lipid peroxidation was assessed using the Thio barbituric acid reactive substances (TBARS) assay to quantify malondialdehyde (MDA) levels. Fourier-transform infrared (FTIR) spectroscopy was used to detect molecular alterations in lipid structure. TBARS results showed no statistically significant differences in MDA levels between DEP-exposed and control groups. In contrast, FTIR spectroscopy revealed consistent shifts in the carbonyl region across all DEP groups ($p = 0.026$), indicating early structural changes in liver lipids. Additional spectral data showed modest increases in nucleic acid signals, especially in exposed animals. These outcomes suggest that even short-term DEP exposure during early life may initiate subtle, early-stage liver changes, supporting the importance of molecular monitoring for detecting pollutant-related health effects before clinical symptoms appear. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475, NIEHS award number R15ES035973 and the USDE Title V DHSI PR/ Award number P031S230161.

Isolation of Outer Membrane Vesicles as the Potential Method of Transport of the Bacterial Genotoxin Colibactin

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Colibactin is a bacterial genotoxin that is produced in certain strains of Gram-negative bacteria by a group of enzymes encoded in the *pks* gene island. The presence of *pks+ E. coli* has been linked to a predisposition to early onset of colorectal cancer. It has been hypothesized that the mechanism by which colibactin gets into

the cells of the digestive tract is via a transit system based on outer membrane vesicles (OMVs). OMVs are liposomes that are secreted from the outer membrane of Gram-negative bacteria and are used to transport different metabolites, including toxins. This project aims to isolate these outer membrane vesicles from different E. coli bacterial strains and use mass spectrometry to gauge any chemical differences among them. So far, we have isolated and quantified OMVs, and their quantities are on par with the typical yields. Extraction of the OMV metabolites will be done using two protocols (i.e., C18 columns, AMICON 100K filtration) to confirm the presence of known metabolites related to colibactin. The suite of experiments to be performed includes but is not limited to SDS-PAGE, reverse phase HPLC and mass spectrometry (e.g, Q-ToF, MALDI-ToF). This analytical exploration of OMVs may ultimately determine compounds linked to the effects of colibactin on the cells of the digestive tract. **Funding**: Supported by the NIH Grant P20GM103434 to the West Virginia IdeA Network for Biomedical Research Excellence & NIH Grant P20GM103475 to the Puerto Rico IdeA Network for Biomedical Research Excellence.

Expression and Purification of the Cytoplasmic Tail of Wsc2p, a Domain Directly involved in the CWI Pathway, for Future Crystallographic Studies

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Through recent studies the Cell Wall Integrity (CWI) pathway has been associated with the rise of antifungal resistance in pathogenic fungi. Due to the various clinical implications this poses to immunocompromised patients, the initiative to develop new inhibitors that can bypass this defensive pathway has become a major opening for research. In the model organism *Saccharomyces cerevisiae*, the mechanosensory Wsc2p has been found to function as an activator of the CWI pathway. Most notably, its cytoplasmic tail can interact with the small GTPase Rho1p, which in turn activates mitogen-activated protein kinases that induce the expression of enzymes that synthesize β-1,3-glucan. However, the limited crystallographic data pertaining to the cytoplasmic tail has delayed the development of new drugs that could inhibit this signaling cascade. As such, the objective of this project is to express and purify the cytoplasmic tail of Wsc2p (Wsc2p CT) for crystallographic analysis, with the goal of elucidating its three-dimensional structure and, in turn, facilitating the design of new inhibitors in the CWI pathway. To achieve this, a DNA fragment encoding the cytoplasmic tail was extracted from the *S. cerevisiae* genome and ligated into an expression vector containing a polyhistidine tag (6xHis tag). Additionally, a synthesized X-factor cleavage site oligonucleotide was added to the vector to enable the removal of the 6xHis tag after subsequent purification and characterization of the peptide. Once expressed and isolated, the peptide can be submitted for crystallographic screening to determine optimal growth conditions for single-crystal X-ray diffraction and thus elucidate the three-dimensional structure of Wsc2 CT. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Theme 4: Public Health, Psychology, and Community-Engaged Research

Students in this theme explored the interplay between mental health, lifestyle, and social well-being. Projects included behavioral studies on problematic gaming among adolescents, analysis of microbiome differences influenced by lifestyle factors, and community-based science education through the Semilla program. Together, these studies illustrated the role of culturally responsive mentorship and community engagement in promoting public health research and emotional wellness among underrepresented populations.

Preliminary Analysis of Loneliness, Coping, and Emotional Psychopathology as Predictors of Problematic Video Game Use Disorder in Adolescents

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El uso excesivo de videojuegos ha desarrollado preocupación en el campo de la psicología, especialmente entre adolescentes, debido al desarrollo potencial del Trastorno de Juego por Internet (IGD) (Gao et al., 2022). Este trastorno no puede entenderse como un simple hábito de ocio, sino como una manifestación compleja que interactúa con múltiples variables psicológicas, los mecanismos de afrontamiento y soledad. En el contexto puertorriqueño, la exploración de estos factores resulta especialmente relevante dada la necesidad de instrumentos culturalmente válidos y de modelos de intervención adaptados a las realidades de los jóvenes en la isla. Esta investigación explora la relación entre el IGD y diversos factores psicológicos (ansiedad, depresión, mecanismo de afrontamiento) y soledad. Se aplicó un diseño transversal con muestreo por conveniencia, utilizando instrumentos válidos en español (IGDS9-SF, DASS-21, COPE Breve, SESLA-S). La muestra estuvo compuesta de XXXP participantes que completaron una encuesta en línea entre las edades de 13 y 19 años. Los resultados del análisis de correlación de Pearson revelaron correlaciones significativas de magnitud moderada a alta entre el IGD y estrés (r=.63), depresión (r=.57) y ansiedad (r=.53), además de asociaciones positivas con estrategias de afrontamiento adaptativas y evitativas. No se encontraron correlaciones significativas con la soledad, lo que sugiere factores contextuales protectores. Se concluye que el IGD como un trastorno transdiagnóstico vinculado al malestar emocional. Hay factores subyacentes que ocurre antes de la presentación de IGD, lo cual pueden influir en su desarrollo. Estos hallazgos destacan la importancia de intervenciones que fomenten la regulación emocional y afrontamiento saludable en adolescentes que presentan conductas conflictivas de juego digital.
Funding: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475 and NIH/COBRE under grant number 1P20GM103642.

Lifestyle Factors may impact the Oral And Cervicovaginal Microbiome of Women Living in Puerto Rico

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Various exogenous and endogenous factors –including cigarette and alcohol consumption, body mass index (BMI), and sexual behavior– can alter the oral and vaginal microbiome. However, little is known to what extent the bacterial and fungal communities can be shifted by these lifestyle factors in the Puerto Rican population. Hence, a matched meta-analysis approach was conducted to evaluate data across two studies and identify microbial patterns associated with these lifestyle factors. Women 21-49 years old underwent collection of posterior fornix

swabs (IRB Streamlyne #2290033153) and saliva samples (IRB protocol 2018-01-01). Projects had genomic DNA extracted for 16S rRNA and ITS-1 amplification and gene sequencing. Taxonomies were described with the Greengenes2 extended library for bacterial taxa (n=87; 47 cervical and 40 oral), while for fungal identification, the UNITE 8.0 database was used (n=84; 47 cervical and 37 oral). High-risk behaviors—such as smoking, drinking, and multiple sex partners— were associated with distinct bacterial communities in both oral and cervical samples. BMI did not significantly impact microbial composition nor diversity in either body site. The fungal composition was altered in the microbiomes of patients who engaged in medium- and high-risk behaviors. Specifically, high-risk behavior was associated with greater fungal richness, and Basidiomycota was identified as a biomarker for these patients. Although BMI did not significantly change microbial composition, lifestyle- associated risk behaviors do influence bacterial structure in the oral microbiome of Hispanic women. High-risk behaviors also influenced the richness of fungal species in patients, while low- risk behaviors influence fungal diversity. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475. Funding support was also provided by the NIH Center for Collaborative Research in Minority Health and Health Disparities (RCMI) 2U54MD007600 and the Puerto Rico Clinical and Translational Research Consortium (PRCTRC) U54MD007587.

Semilla Community: Empowering Childhood Wellness Through Science

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The Semilla Community is an experiential educational initiative born from a partnership between the Boys & Girls Club of Puerto Rico (BGCPR). Designed for children ages 9–12 from underrepresented communities, Semilla fosters resilience and whole-person health through transdisciplinary learning. Grounded in the eco-bio-developmental (EBD) model, the program addresses the impact of toxic stress and adverse childhood experiences on academic success, emotional well-being, and social development factors that often discourage engagement in STEM fields. Semilla promotes a health agency by helping children express personal needs and connecting science, education, medicine, and community. The Social-Emotional Learning (SEL) module helps participants understand stress and its impact on mental and physical health while exploring strategies for emotional regulation. Implemented in three BGCPR Clubs, the SEL curriculum spans six days and combines psychosocial learning with scientific inquiry. Activities include interactive discussions on various types of stress, mindfulness techniques such as breathing and relaxation exercises, and a zebrafish observation experiment to explore biological stress responses. Evaluation tools included the Spanish-School Science Attitude Survey (S-SSAS), program-specific feedback forms, and informal caregiver interviews. Twenty-seven participants completed SEL activities. Quantitative data showed high enjoyment (mean score: 4.44/5) and perceived gains in emotional awareness and scientific engagement. While S-SSAS pre-post scores did not yield statistical significance, qualitative responses highlighted increased curiosity, self-confidence, and application of stress-reducing strategies at home and school. Caregivers reported observable improvements in behavior and well-being. Semilla's SEL curriculum effectively engages children in understanding and managing stress while cultivating scientific thinking. Its integration of science, emotional wellness, and community-based feedback supports holistic development and positions SEL as a vital tool for educational equity and childhood empowerment in underrepresented populations. **Funding**: This research was supported by the Institute of General Medical Sciences Grant #1R25GM137368-01A1, and partially supported by The Hispanic Alliance for Clinical and Translational Research Award number U54GM13380.

Theme 5: Biotechnology, Bioengineering, and Computational Biology

This theme highlighted student innovation in technology development and computational modeling. Projects featured the creation of wearable biosensors for neurotransmitter detection, protein modeling for cancer targets, and integrative proteomic and metabolomic analyses. Students leveraged engineering, bioinformatics, and molecular tools to advance biomedical diagnostics, data interpretation, and translational applications.

Flexible Electrochemical Sensor for the Detection of Neuropeptide Y in Human Sweat

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Inkjet printing technology has greatly benefited the development of wearable devices. The use of conductive inks and an inkjet printer makes possible the design of electrodes by depositing the conductive material on the surface of a substrate. The development of wearables for sweat analysis has been of great interest since the same physiological information obtained in blood can be analyzed in sweat. Also, sweat biomarker analysis is a non-invasive sampling, is more cost-effective than blood analysis, and can analyze biomarkers in real time. Our research group has successfully manufactured a prototype of paper-based electrochemical device using silver inkjet electrodes. We have managed to detect different concentrations of Neuropeptide Y (NPY) in artificial sweat through square wave voltammetry using silver inkjet electrodes printed on PET, Kapton and TPU. NPY is a 36 amino acid neuropeptide associated with different diseases and conditions, such as epilepsy, Parkinson's disease, and anxiety. Although we detected concentrations of NPY in artificial sweat electrochemically, the change in pH in sweat and the long exposure of silver inkjet electrodes in solution affected the electrochemical measurement. To improve the performance of silver inkjet electrodes, the surface of the silver inkjet electrodes was modified with onion-like carbon (OLC) particles. Silver inkjet electrodes coated with OLC resulted in better stability and performance in the electrochemical detection of NPY concentrations in artificial sweat at pH 4, 6.5, and 8. By modifying the surface of silver inkjet electrodes with OLC, we detected NPY concentrations as low as 1 pg/mL NPY in artificial sweat. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Development of an Electrochemical Biosensor for the Detection of Serotonin in Sweat Using Metallic Alloys

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Serotonin is an important neurotransmitter that regulates various functions of the central and enteric nervous systems, as well as diverse physiological processes. The abnormal release of serotonin has been associated with medical conditions, including mood disorders such as depression and certain types of cancer like carcinoid tumors. Current methods for measuring serotonin are often expensive, requiring specialized equipment and trained personnel. In addition, the interference of structurally similar molecules such as dopamine presents challenges for selective detection. This experiment focuses on developing a serotonin biosensor using metallic catalytic alloys, reduced through chemical synthesis in an emulsion system. $NaBH_4$ was used as a reducing agent, with metal salts dissolved in the aqueous phase. The alloys were formulated from copper (Cu), cobalt (Co), and nickel (Ni), and were used to modify glassy carbon electrodes (GCE) through the drop-casting technique. Electrochemical studies were conducted to identify the oxidation potential of serotonin and evaluate the selectivity of the electrode in the presence of common interferents such as dopamine. The electrode modified with the CoCu alloy showed oxidation potentials different in comparison to the unmodified control. For example,

CoCu showed serotonin oxidation approximately at 0.421 V vs Ag/AgCl, while unmodified serotonin oxidized at 0.375 V vs Ag/AgCl. The CoCuNi alloy, evaluated through square wave voltammetry (SWV), clearly separated the peaks of dopamine (~0.210 V) and serotonin (~0.500 V), avoiding overlaps. Additional studies will be conducted to evaluate the performance and specificity of neurotransmitter biosensors like this one for future applications. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Evaluating Automated Brain Cell Detection Performance against Manual Counting in DAB-Stained Non-Human Primate Tissue

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Accurate identification of neurons in brain tissue is critical for evaluating the effectiveness of viral transfection techniques, particularly in non-human primates (NHPs), where transfection success can vary due to differences in promoters, tags, and other factors. This project aimed to assess transfection outcomes—for example cell density and cell type distribution—across different brain regions, experimental conditions, and transfection strategies. Although manual cell counting is highly accurate, it is time-consuming and impractical for large-scale analyses. To address this, we validated an automated neuron detection system developed in QuPath for identifying DAB-stained neurons in macaque brain slices. The system's performance was evaluated by comparing automated detections to manual annotations using spatial (Euclidean distance) and morphological (intersection over union) criteria. Detections were classified as true positives (TP), false positives (FP), or false negatives (FN), and standard performance metrics—including sensitivity, precision, and F-score—were calculated. The results demonstrate that the automated system reliably detects and quantifies neurons, ensuring that observed differences in cell density or morphology across conditions reflect true biological variation rather than detection artifacts. This approach enables efficient evaluation of transfection outcomes and supports broader implementation in both neuroscience research and clinical applications. **Funding**: University of Wisconsin-Madison, Dr. Ari Rosenberg's Lab, Medical Scientist Training Program, Neuroscience Training Program, Wisconsin National Primate Research Center, MATLAB, QuPath.

Elucidating the Role of IFN-I in Modulating MDSCs Glycolysis Using an In Vitro Model

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Cancer is a leading cause of death in Puerto Rico and the United States. A key feature of cancer progression is the expansion of myeloid-derived suppressor cells (MDSCs), which suppress immune responses, promote tumor growth, and contribute to immunotherapy resistance. MDSCs reprogram their metabolism, particularly shifting toward glycolysis, to support their immunosuppressive functions. Type I interferons (IFN-I) have been shown to reduce the immunosuppressive activity of MDSCs, but the mechanisms by which they regulate MDSC

metabolism remain unclear. To investigate this, we developed an in vitro model of MDSC-like cells (iMDSCs) using HL-60 promyelocytic cells differentiated with GM-CSF and IL-6. Flow cytometry analysis confirmed successful generation of iMDSCs, as shown by increased CD11b expression. We then compared the expression of immunosuppressive genes between undifferentiated HL-60 cells and iMDSCs. Notably, Ptges was upregulated in iMDSCs, while Arg1 and Nos2, as reported in the literature, were not. This finding is novel in the context of this in vitro model. Next, we evaluated two glycolytic genes, GLUT1 and PKM, and found no significant changes between HL-60 and iMDSCs. Treatment of iMDSCs with IFN-α also failed to alter the expression of these glycolytic genes. Our findings support the reproducible generation of MDSC-like cells in vitro and reveal the upregulation of Ptges, suggesting alternative immunosuppressive mechanisms may be at play. Future studies will expand gene profiling to better understand metabolic regulation by IFN-I and validate findings in clinical samples. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Integrative Proteomic and Metabolomic Analysis Reveals GRP78 as a Driver of Stress Adaptation in GBM

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Glioblastoma multiforme (GBM) is one of the most aggressive and treatment-resistant primary brain tumors of the central nervous system. Endoplasmic reticulum (ER) stress plays a pivotal role in tumor cell survival and metabolic adaptation. Glucose-regulated protein 78 (GRP78), a key ER chaperone, is central to maintaining proteostasis under stress conditions and has been implicated in tumor progression. This study examined GRP78 expression and downstream stress-related pathways using the A172 GBM cell line, GBM tumor tissues, and patient-derived xenograft (PDX) models. Western blotting confirmed elevated GRP78 levels in A172 cells and GBM tissues compared to normal brain. TUNEL assays indicated increased apoptosis following treatment with the GRP78-targeting antibody PAT-SM6. Untargeted proteomic profiling identified metabolic shifts in PAT-SM6-treated cells, including altered ER stress markers and enhanced glycolytic and glutamine metabolism. NMR-based metabolomic analysis further revealed specific pathway changes, including increases in lactate and serine levels. Collectively, this work underscores GRP78's role in promoting tumor growth and stress-adaptive metabolic flexibility in GBM. The integration of molecular techniques such as Western blotting, qRT-PCR, proteomics, and metabolomics provided a comprehensive view of GRP78-mediated pathways in glioblastoma progression. GRP78 emerges as a critical node in tumor survival and metabolism and may offer a promising molecular target for future therapeutic strategies. **Funding**: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

Theme 6: Translational and Collaborative Research (U54 & PR-INBRE Partnerships)

The final category emphasized collaborative and translational studies supported through PR-INBRE and U54 partnerships with institutions such as the University of Texas MD Anderson Cancer Center and the University of Wisconsin–Madison. These projects examined immunotherapeutic strategies, virotherapy optimization, and molecular pathways influencing tumor immunity and treatment response. Collectively, they demonstrated the impact of multi-institutional collaboration in advancing cutting-edge biomedical research and building scientific leadership among Puerto Rican trainees.

Targeting Immunosuppressive JAK-IDO1 Pathway to Strengthen Oncolytic Virotherapy in Glioblastoma

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Most patients with glioblastoma die within a year after diagnosis, underscoring the urgent need for innovative therapeutic strategies. Oncolytic viruses are emerging as a promising treatment approach. In our lab, we developed Delta-24-RGD, an oncolytic adenovirus that has demonstrated promise in clinical trials for patients with recurrent glioblastoma (NCT00805376, NCT02798406). These clinical studies, together with preclinical data, showed that Delta-24-RGD's efficacy stems from direct tumor cell oncolysis and indirect activation of anti-tumor immunity. To further leverage this immune component, our group generated Delta-24-RGDOX, a next-generation virus that expresses the T-cell activator OX40-L. Here, we aim to improve the efficacy of Delta-24-RGDOX by targeting factors that maintain the immunosuppressive characteristic of gliomas. Specifically, the IFNγ-JAK-IDO1 pathway. We performed bulk RNA sequencing of murine gliomas treated with Delta-24-RGDOX or control (PBS) and analyzed the upstream regulators using Ingenuity Pathway Analysis. We also analyzed patient tumor biopsies from Delta-24-RGD plus pembrolizumab clinical trial (GSE226976) and assessed IFN-IDO1 correlation using R. IFNγ-induced IDO1 expression was validated by western blots and qPCR in glioma cells. To study macrophage polarization, bone marrow-derived macrophages (BMDM) were stimulated with IFN in the presence or absence of Ruxolitinib (a clinically approved JAK inhibitor), and gene expression of immunosuppressive markers (Retnla, Arg1, CD274 (PD-L1), & IDO1) was assessed by qPCR. Bulk RNA-seq analysis revealed IFNγ as the top upstream regulator. In clinical samples, IFNγ and IDO1 expression were strongly correlated, supporting clinical relevance of this axis. In vitro, IFNγ robustly induced IDO1 expression in glioma cells, whereas Ruxolitinib reverses this effect. Ruxolitinib treatment also reduced expression of anti-inflammatory genes in IFN-polarized BMDMs, indicating a shift away from an immunosuppressive phenotype. These findings suggest that IFNγ-driven IDO1 upregulation may impair the efficacy of oncolytic virotherapy, and combining it with JAK inhibitors enhances therapeutic benefit. Our study provides a rationale for combining oncolytic virotherapy with JAK inhibition to overcome resistance in gliomas. **Keywords**: glioblastoma, oncolytic virus, IFNγ-JAK-IDO1 pathway, immunosuppression. **Funding**: U54 Partnership for Excellence in Cancer Research.

The Impact of Immune Surveillance on Tumor Growth in Aged, Genetically Matched Oral Cancer Models

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Non-HPV associated head and neck squamous cell carcinomas (HNSCC) frequently arise from oral premalignant lesions due to the accumulation of genetic mutations and exposure to carcinogens such as tobacco, alcohol, and aging. To investigate mechanisms of immune evasion and immunotherapy resistance, we developed a panel of syngeneic oral cancer cell lines derived from aged Trp53/Cdkn2a mutant mice. Among these, ROC14A and

ROC14B derived from the same animal display markedly different tumorigenic capacities. We hypothesize that differences in T cell–mediated immune surveillance contribute to their divergent in vivo behavior. ROC14A and ROC14B cells were orthotopically implanted into both immunodeficient RAG1 knockout and immunocompetent C57BL/6J mice. To assess the role of cytotoxic T cells, a subset of immunocompetent mice received anti-CD8 antibody treatment. Cell lines were evaluated by western blot to determine the levels of mutant p53, cytokeratin and mesenchymal markers. The tumor immune microenvironment was analyzed by immunohistochemistry (IHC), and gene expression profiles were evaluated by quantitative PCR. ROC14B tumors grew robustly in CD8-depleted mice, indicating that $CD8^+$ T cells actively suppress its growth in immunocompetent hosts. IHC revealed that most ROC tumors exhibit high proliferative indices and limited lymphocytic infiltration, with macrophages being the predominant immune cell type. Western blot analyses showed variable expression of epithelial and mesenchymal markers, including elevated vimentin in ROC14 and increased keratin-14 in ROC15. The ROC tongue tumors expressed immunosuppressive cytokines and checkpoint molecules, along with evidence of M2-polarized tumor-associated macrophages, suggesting a tumor microenvironment that supports immune evasion and contributes to resistance to current immunotherapies. Despite their shared genetic origin, ROC14A and ROC14B display differential susceptibility to immune surveillance, likely due to the presence of neoantigens in ROC14B that trigger $CD8^+$ T-cell–mediated tumor control. The ROC cell line panel recapitulates key features of the immunosuppressive HNSCC microenvironment and provides a valuable platform to study immune escape mechanisms and to develop next-generation immunotherapeutic strategies. **Keywords**: Head and neck squamous cell carcinomas, syngeneic mice, aging, immune surveillance. **Funding**: U54 Partnership for Excellence in Cancer Research.

Targeting TRIP13 in Cancer: Exploring Small Molecule Inhibitors in HPV-related Models

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Human papillomavirus (HPV) is responsible for all cases of cervical cancer and a significant subset of head and neck squamous cell carcinomas, particularly oropharyngeal cancers, represent 5% of global cancer incidence and lack targeted, effective therapies. Previous research demonstrated that in HPV+ cancer cells, the inhibition of the ATPases Associated with diverse cellular Activities (AAA ATPase) Thyroid Hormone Receptor Interactor 13 (TRIP13) through knockdown combined with the Aurora kinase A inhibitor alisertib induced apoptosis. TRIP13 has also been found to be overexpressed in numerous cancers and has been correlated with poor prognosis and therapeutic resistance. We hypothesize that co-inhibition of TRIP13, and Aurora kinase A could provide a novel strategy for treating HPV-related cancers. We evaluated the efficacy and potency of a known TRIP13 inhibitor (DCZ0415) and three in-house novel compounds (T62, T77, T92) using dose-response and combination treatment assays in human (HeLa, ME180) and murine (TC-1, C3.43) cell lines. Cell viability was assessed in a dose kinetics with single agent treatment (TRIP13 inhibitors) via CellTiter-Glo Assay for 72hrs. Additionally, selected dose (10uM and 20uM of TRIP13 inhibitors and alisertib (100 and 200nM) to address combination treatment efficacy. Apoptosis was quantified via annexin V/PI staining, and immunoblotting was performed to validate apoptotic hallmarks. In murine cell lines, DCZ0415 reduced cell viability more effectively than in-house TRIP13 inhibitors, though combining inhibitors with alisertib did not enhance efficacy, and annexin V/PI assays showed no significant increase in apoptosis. IB data confirmed apoptotic hallmarks observed in the annexin data. Similarly, in C3.43 cells, single-agent DCZ0415 was more effective than combination therapy, with minimal apoptotic response observed for all treatments. In human cell lines especially ME180, T62 alone displayed minimal toxicity; however, combining T62 with alisertib led to a marked induction of apoptosis, highlighting the potential of this combination strategy for HPV-associated cancers. HeLa cells also exhibited a similar pattern,

showing enhanced apoptotic response upon combination treatment. Three novel TRIP13 inhibitors were tested in HPV+ models, with T62 showing promising apoptotic synergy when combined with alisertib despite modest effects on viability. Future work will focus on elucidating the exact cell death mechanisms, optimizing inhibitor potency and specificity, and evaluating combination therapies in Rb-deficient cancers. **Funding**: U54 Partnership for Excellence in Cancer Research.

Cardiac-specific Protein Profiles Analysis in Undifferentiated AC-16 Cells exposed to Xylazine-induced Toxicity

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Xylazine, a veterinary tranquilizer increasingly misused as an adulterant in illicit opioids, has been associated with endothelial damage and suspected cardiotoxic effects. As part of an ongoing investigation into xylazine-induced cardiac damage, we evaluated the baseline expression of cardiomyocyte-associated proteins in AC-16 human ventricular fibroblasts prior to differentiation. Cells were treated for 24 hours with vehicle (negative control, NC), camptothecin (positive control, PC, 70 ng/mL), or xylazine (XYL, 70 ng/mL). Total protein was extracted and quantified via the Bio-Rad DC Protein Assay Kit, yielding concentrations of 99.62 µg/mL (NC), 69.39 µg/mL (PC), and 314.77 µg/mL (XYL). Western blotting was performed using 20 µg of total protein per lane to assess the presence of CK-MB, cardiac troponin T, myoglobin, and GAPDH (loading control). Two mouse monoclonal antibodies targeting CK-MB (ab404 and ab19603) were used. CK-MB was detected in all samples at ~40 – 43 kDa using IRDye 680RD anti-mouse. Cardiac troponin T (ab10214) was not conclusively detected and Myoglobin (~17 kDa), probed with rabbit monoclonal antibody, using IRDye 800CW anti-rabbit, was not detected. GAPDH was consistently detected at ~37 kDa in all conditions. These preliminary findings suggest that undifferentiated AC-16 cells exhibit partial cardiac protein expression, including CK-MB, but lack others such as myoglobin, consistent with an immature cardiomyocyte phenotype characteristic of untransformed AC-16 cells. Future studies will involve repetition using 30 µg of total protein, statistical analysis with biological replicates, and comparison with differentiated AC-16 cardiomyocytes. **Funding**: This research was partially supported by the following programs the RCMI Program (U54 MD007600) from the National Institute on Minority Health and Health Disparities (NIMHD), the NIH-R25 Hispanic Clinical and Translational Research Education and Career Development (HCTRECD) Award (R25MD007607), the Hispanics in Research Capability (HiREC) Endowment Award (S21MD001830) from the NIMHD, and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.