
UNIVERSITY OF PITTSBURGH
SEVENTEENTH
POSTDOCTORAL
RESEARCH
SYMPOSIUM
April 15, 2024 | 2-5pm
William Pitt Union

UPPDA

University of Pittsburgh Postdoctoral Association

April 15, 2024

Dear Colleagues,

Welcome to the 17th Postdoctoral Research Symposium, formally known as the Data & Dine event.

We dedicate this event to both past and future postdoctoral fellows at the University of Pittsburgh. The Symposium serves as a celebration of the remarkable research contributions made by postdoctoral fellows at Pitt. We are honored to have you participate and share your insights, expertise, and passion for advancing knowledge in your respective fields.

We hope this symposium continues to provide a forum of support and networking for postdoctoral fellows across the University. We encourage you to visit other posters, share contact information, and get to know your fellow postdocs. Additionally, please note that elections for the UPPDA board will be happening over the next month, so please stop by and chat with us if you are interested in getting involved!

We are delighted to continue our tradition of awarding professional development awards for best poster presentations. Thanks to the generous support of our academic community, the University of Pittsburgh Postdoctoral Association will be recognizing 10 postdoctoral fellows with professional development awards.

This event would not have been possible without the help of the Office of Academic Career Development, and financial support from the Office of the Senior Vice Chancellor for the Health Sciences, the Office of the Provost, and departmental sponsors. We would also like to extend our thanks to the poster judges and other volunteers contributing to this event's success.

And finally, we thank fellows, faculty, and administrators for sharing this wonderful day with us!

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AGENDA
April 15, 2024 | 2:00-5:00 PM
WILLIAM PITT UNION

12:00-2:00 PM	**Poster Set-Up & Check-in** Lobby
2:00-2:45 PM	**Poster Session 1** Odd Numbered Posters Ballroom and Kurtzman Room
2:45-3:30 PM	**Poster Session 2** Even Numbered Posters Ballroom and Kurtzman Room
3:30-5:00 PM	**Networking & Awards Reception** Assembly Room

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POSTDOCTORAL ALUMNI AWARD WINNER

Dr. Mohammadreza Tabary

Dr. Tabary is a dedicated physician-scientist specializing in lung immunology. He completed his medical education at Tehran University of Medical Sciences before relocating to the United States in 2021 to advance his career in medicine and research.

Currently serving as a Postdoctoral Associate within the Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine (PACCSM) at the University of Pittsburgh, Dr. Tabary focuses his research on elucidating the intricate mechanisms of lung immunology. His work aims to advance our understanding of respiratory diseases and the interactions between hosts and pathogens.

Dr. Tabary is poised to begin the next phase of his medical journey as he starts his Internal Medicine Residency at Baylor College of Medicine. With a steadfast dedication to patient-centered care, research excellence, and advocacy for underserved patient populations, Dr. Tabary is committed to making meaningful contributions to the field of medicine and positively impacting the lives of individuals from all walks of life.

Outside of his medical pursuits, Dr. Tabary finds joy in playing the piano and has a passion for computer programming.

ABOUT THE POSTDOCTORAL ALUMNI AWARD The University of Pittsburgh Postdoctoral Association offers an annual Postdoctoral Alumni Award to an individual who has demonstrated a profound, sustained, or leadership contribution to the Postdoctoral Association. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

Past Alumni Award Recipients

2023 – David Gau, PhD
2019 – Kimberly Payne, PhD
2018 – Chelsea Stillman, PhD
Collin Diedrich, PhD
2017 – Karen Carney, PhD
2016 – John Merriam, PhD
2014 – Catherine Haggerty, PhD
2013 – Timothy Maul, PhD
2012 – Aaron Bell, PhD
2011 – Natacha De Genna, PhD
2010 – Lei Hong, PhD
2009 – David Robinson, PhD
2008 – Richard Bodnar, PhD
2007 – Steven Wendell, PhD
2006 – Elsa Strotmeyer, PhD
2005 – Stuart Olmsted, PhD

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POSTDOCTORAL ADVOCATE AWARD WINNER

Dr. Ian Sigal

Dr. Sigal received a B.Sc. in Physics from UNAM in México City (1999), a M.A.Sc. in Aerospace Engineering from the University of Toronto, Canada (2001), and a Ph.D. in Mechanical Engineering also from the University of Toronto, Canada (2006). Dr. Sigal has dedicated his scientific career to the study of the eye biomechanics and glaucoma. In 2010 he joined the University of Pittsburgh and founded the Laboratory of Ocular Biomechanics. He has pioneered methods for experimental and computational study of eye microarchitecture and mechanics. Current projects include methods to measure eye mechanics in vivo and in situ, and to evaluate the interplay between connective, vascular and neural tissues in eye disease. He has over 125 peer-reviewed manuscripts and a Google scholar h-index of 48. His research is supported by the National Institutes of Health, the Canadian Institutes of Health Research, the Glaucoma Research Foundation, Research to prevent blindness and other organizations.

ABOUT THE POSTDOCTORAL ADVOCATE AWARD The University of Pittsburgh Postdoctoral Association offers an annual Postdoctoral Advocate Award to an individual who has demonstrated a profound, sustained, or leadership contribution to the Postdoctoral Association. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

Past Advocate Award Recipients

2023 – Amanda Godley, PhD
2019 – Nathan Urban, PhD
2018 – Thomas Smithgall, PhD
2017 – Arvind Suresh, MS
2016 – Jennifer Woodward, PhD
2015 – Ora Weisz, PhD
2014 – Alan Sved, PhD
2013 – Charles Nieman, PhD, DSO, ARO
2012 – Darlene Zellers, PhD
2011 – Christopher Martin, PhD
2010 – Patricia Beeson, PhD
2009 – Bruce Freeman, PhD
2008 – Andrew Blair, PhD
2007 – Joan Lakoski, PhD
2006 – Elizabeth Baranger, PhD
2005 – Arthur Levine, MD

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POSTDOCTORAL MENTOR AWARD WINNER

Dr. Michele Levine

Michele D. Levine, PhD, a licensed clinical and health psychologist, is Professor of Psychiatry, Psychology and Obstetrics, Gynecology and Reproductive Sciences at the University of Pittsburgh. Dr. Levine's program of research which has been funded by the National Institutes of Health since 2002, focuses on relationships among health behaviors and mental health during pregnancy and the postpartum period. For example, currently projects include a sequential multiple assignment randomized trial evaluating the sequence of lifestyle interventions to optimize maternal cardiometabolic health at one year postpartum among pregnant participants with prepregnancy obesity, a study examining the roles of impulsive phenotypes in perinatal weight, and one designed to determine the relationship lifetime stress exposure and cardiovascular disease risk in Black pregnant women.

Dr. Levine also directs a T32 postdoctoral training grant and an affiliated clinical psychology training program at Western Psychiatric Hospital, both of which support advanced training for junior clinical scientists. In addition to the professional fulfillment these training roles provide, Dr. Levine has derived great joy from serving as a primary mentor to more than 10 postdoctoral scholars and co-mentor to several others, as well as from serving on thesis and dissertation committees, sponsoring undergraduate directed research study students, and supporting medical students and residents interested in maternal health behavior and the career development of junior colleagues pursuing clinical licensure in psychology.

ABOUT THE POSTDOCTORAL MENTOR AWARD The University of Pittsburgh Postdoctoral Association Mentor Award criterion is modeled from the National Postdoctoral Association (NPA) Mentor Award, which recognizes a faculty member who has been engaged in exceptional mentoring of postdoctoral fellows and postdoctoral scholars. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

Past Mentor Award Recipients

2023 – Ian Scott, PhD
2019 – Ronald Buckanovich, PhD
2018 – Tina Goldstein, PhD
2017 – Brooke Molina, PhD
2016 – Melissa Bilec, PhD
2015 – Jennifer Silk, PhD

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POSTER PRESENTATION & AWARD GUIDELINES

Posters will be judged from 2 PM to 3:30 PM.
Presenters with odd numbered posters will be judged from 2 PM to 2:45 PM.
Presenters with even numbered posters will be judged from 2:45 PM to 3:30 PM.
Presenters are to be present at their posters at their assigned times.

The top poster presenters will each receive a $500 Professional Development Award. These awards will provide funds for professional development, including participation in a scientific conference, and are to be used by recipients between July 2024 and May 2025. The award categories include:

- *Basic Biomedical and Pharmacological Sciences*
- *Behavioral Sciences*
- *Cellular and Molecular Biology*
- *Clinical and Surgical Sciences*
- *Engineering, Physical, and Computational Sciences*

All posters have been assigned to one of these categories for judging purposes. The judges will not be identified during the poster sessions. Scoring will be based upon creativity, style, content, impact, presentation, and overall impressions.

Award winners will be announced during the reception following the poster sessions.

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POSTER PRESENTATION AWARD SPONSORS

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School of Medicine

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Pittsburgh Institute of Neurodegenerative Diseases

The Center for Military Medicine Research

BRONZE SPONSORS
$0 - $250

Department of Bioengineering
Swanson School of Engineering

Department of Cell Biology
School of Medicine

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Poster 94

Plasma GFAP changes as a secondary endpoint in clinical trials

Sarah Abbas[1], Pamela C. L Ferreira[1], Bruna Bellaver[1,2], Guilherme Povala[1,2], Francielli Rohden[1], Cristiano Schaffer Aguzzoli[1], Hussein Zalzale[1], Guilherme Negrini[1], Carolina Soares[1], João Pedro Ferrari-Souza[2], Douglas T. Leffa[1], Firoza Z. Lussier[1], Matheus Rodrigues[1], Markley Silva[1], Cynthia Felix[1], Emma Ruppert[1], Pampa Saha[1], Marina Madeiros[1] Cécile Tissot[3] , Joseph Therriault[3], Helmet Karim[1], Chang Hyung Hong[4], Hyun Woong Roh[4], Thomas K. Karikari[1], Eduardo R Zimmer[2], Pedro Rosa-Neto[3] , Sang Joon Son[4] Tharick Pascoal[1]

[1]Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA [2]Graduate Program in Biological Sciences: Biochemistry, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. [3]Translational Neuroimaging Laboratory, McGill University Research Centre for Studies in Aging, Alzheimer's Disease Research Unit, Douglas Research Institute, Le Centre intégré universitaire de santé et de services sociaux (CIUSSS) de l'Ouest-de-l'Île-de-Montréal [4]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

Background: Cerebrospinal fluid (CSF) and positron emission tomography (PET) amyloid-beta (Aβ) and tau measures have been used as endpoints in several clinical trials in AD. Recently, clinical trials have incorporated plasma markers as endpoints, including glial fibrillary acidic protein (GFAP). Here, we tested the characteristics of longitudinal changes in plasma GFAP as a secondary or exploratory endpoint of clinical trials focusing on cognitively unimpaired (CU) or impaired (CI) patients.

Methods: We assessed 264 individuals (71 CU (25% Aβ positive), 107 CI Aβ positive, and 86 CI Aβ negative) with amyloid-beta (Aβ) positron emission tomography (PET) at baseline and available longitudinal plasma GFAP and amyloid-beta (Aβ) positron emission tomography (PET) at baseline in research-based and memory clinic cohorts. Cox-proportional hazards tested the association between changes in plasma GFAP and cognition. We estimated the sample size needed to test a hypothesized 25% drug effect with 80% power at a 0.05 level on reducing changes in plasma GFAP.

Results: Plasma GFAP showed a greater annualized increase in CU (10–20%) than CI (6–11%) individuals. Changes in plasma GFAP were associated with worsening in the CDR sum of boxes score in research and memory clinic cohorts in the entire population. Clinical trials testing a 25% drug effect in reducing changes in plasma GFAP as a secondary endpoint would require approximately 1,000 subjects per study arm in both cohorts, in CU and CI individuals Aβ positive and negative.

Conclusion: The association of changes in plasma GFAP with worsening cognition suggests that GFAP has the potential to be a significant endpoint for clinical trials. Our results highlighted that longitudinal plasma GFAP can be used as a secondary endpoint in clinical trials in Aβ positive individuals, regardless of cognitive impairment.

Poster 46

A novel approach for sampling brain-derived extracellular vesicles

Alam, Shahnur[1]; Ostach, Mary Ann[1]; Fitz, Nicholas F[1]

[1]Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh PA

Extracellular vesicles (EVs) are nano vesicles secreted into extracellular spaces and contain important cargo including noncoding RNAs (ncRNA). Brain-derived EVs (BdE) are collectively released by all neural cells and may serve as potential biomarkers for disease. However, to gain solid understanding the role of EVs in different conditions, it is crucial to sample and analyse EVs directly from brain interstitial fluid (ISF). Evaluating changes in EVs cargo is extremely difficult due to lack of unique cell-specific markers for circulating EVs in plasma and the difficulty in isolating EVs from tissues such as brain. Furthermore, these assessments provide a single endpoint profiling of EVs cargo and fail to capture potential temporal changes associated with disease progression. Therefore, to better sample and analysis of BdE, novel techniques need to be developed which allow for real time collection from the brain ISF where EVs are directly released. In the present study, we applied a novel method Cerebral Open Flow Microdiyalysis (cOFM) for collecting EVs from the hippocampal ISF (ISF-EV) of awake freely moving mice and subsequently isolated EVs from ISF, plasma and brain tissues in wild-type (WT) and APP/PS mice for compartment specific characterization. Small ncRNA were isolated from the EVs, sequenced and data analyzed using COMPSRA pipeline. We identified a significant number of differentially expressed ncRNA transcripts when comparing ISF-EV and plasma-EV suggesting unique populations of EVs from the two sample types. Further, we determined unique profiles of ISF-EV specific ncRNA transcripts in APP/PS mice compared to WT mice, which are related to amyloid pathology. Thus, the novel cOFM technique represent brain specific circulating EVs and ncRNA from these EVs would be potentially useful as neurodegenerative disease biomarker.

Poster 47

Revealing how CCNE1 oncogene reprograms nuclear metabolism in cancer.

Amandine Amalric[1], Nathaniel W. Snyder[2], Katherine Aird[1]

[1]Department of Pharmacology & Chemical Biology and UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA; [2]Department of Microbiology and Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, USA

Cancers adopt metabolic adaptations for survival and excessive cell division, including differential metabolic compartmentalization allowing cells to maximize certain pathways. However, how oncogenic stress affects nuclear metabolic compartmentalization is unclear. Focusing on a key oncogenic driver, cyclin E1 (CCNE1), which drives aberrant S phase progression, DNA replication, and DNA damage, our data reveal that metabolic reprogramming in CCNE1-driven cells is partly specific to the nuclear compartment. Proteomics on isolated nuclei revealed ~30 metabolic enzymes enriched in the nucleus of CCNE1-driven cells compared to controls, suggesting translocation from the cytoplasm to the nucleus under oncogenic stress. Interestingly, many of these enzymes are related to nucleotide and specialized fatty acid metabolism, typically annotated to the cytoplasm and mitochondria/peroxisome, respectively. In silico analysis identified two candidates with a predicted nuclear localization signal (NLS), namely GMP synthase (GMPS) and Acyl-CoA Synthase Long Chain Family 4 (ACSL4). GMPS is a crucial enzyme in *de novo* purine biosynthesis, whereas ACSL4 is involved in polyunsaturated fatty acid metabolism. These data suggest that oncogenic stress induces translocation of metabolic enzymes, which may directly influence cancer phenotypes. Future studies will determine the role of these enzymes in nuclear metabolic reprogramming of cancer cells using activity assays and metabolomics. Furthermore, we will determine whether NLS affects their nuclear translocation, then test if preventing nuclear localization counteracts chemoresistance. Together, these studies will establish that oncogenic stress drives differential compartmentalization of metabolic enzymes, suggesting development of therapies to target nuclear enzymes or their translocation.

Poster 1

Ultrasound Targeted Microbubble Cavitation (UTMC) for the treatment of Myocardial Microvascular Obstruction (MVO)

Amjad, Muhammad[1]; Mohammed, Soheb[1]; Chen, Xucai[1]; Villanueva, Flordeliza[1]; Pacella, John[1]

[1]Division of Cardiology, Department of Medicine, University of Pittsburgh

Congestive heart failure following acute myocardial infarction is increasing due to microvascular obstruction (MVO), for which there is no effective therapy. We have been developing ultrasound-targeted microbubble cavitation (UTMC) as a potential treatment. Rapacz familial hypercholesterolemic (RFH) pigs were used in this study. MVO was created in the left anterior descending (LAD) microcirculation. UTMC therapy was applied during infusion of Definity®. Left ventricular (LV) segmental wall motion and microvascular perfusion were assessed with ultrasound. Cardiac MRI was obtained to measure infarct size and area of MVO; ultrasound imaging and coronary angiography were performed at 48h. LAD angiographic flow was improved at 48h post-treatment in comparison to control. UTMC treatment significantly improved echo-based LV systolic performance. UTMC was also found to significantly enhance LAD blood volume at 48h versus control. MRI-derived LV segmental wall motion and ejection fraction also improved post-treatment. Infarct size was reduced as assessed by both Evans Blue/TTC staining and MRI. In conclusion, we demonstrated that UTMC significantly reduced infarct size, enhanced LAD microvascular perfusion and improved LV systolic performance.

Poster 33

Predicting Self-Injury Types in Autistic Youth using Machine Learning

Ligia Antezana[1], Caitlin M. Conner[1], Safaa Eldeeb[1], Samuel Turecki[2], Matthew Siegel[3], Helmet T. Karim[1,4], & Carla A. Mazefsky[1]

[1]Department of Psychiatry, University of Pittsburgh School of Medicine;
[2]Cinncinati Children's Hospital Medical Center;
[3]Department of Psychiatry and Behavioral Sciences, Boston Children's Hospital;
[4]Department of Bioengineering, University of Pittsburgh

Self-injurious behavior (SIB) spans a wide range of types (e.g., hitting self, biting self, skin picking). SIB is prevalent in about 42% of autistic youth, yet few studies have disentangled types of SIB, with most literature to date encapsulating all SIB types together. Previous work has linked SIB in autistic individuals with demographics (e.g., younger age), medical problems, and co-occurring psychiatric symptoms. Emerging work has also tied emotion dysregulation to SIB. The study aimed to examine such variables as they relate to severity of SIB types.

Data were derived from the Autism Inpatient Collection, a multi-site comprehensive database on autistic youth who are enrolled in specialized inpatient units for intellectual and developmental disabilities. Data were included from 323 participants [M(SD) age =12.91(3.37) years] whose caregivers completed eight SIB items at admission. Elastic net regressions, which uses machine learning models that perform well with multicollinearity, were used to examine the relationship between each SIB type severity and demographic, medical problems, caregiver report, and emotion dysregulation (reactivity, dysphoria).

The most common predictor of SIB types was greater stereotypic behavior (7/8 types), followed by level of lifetime autistic traits (5/8 types). Demographic predictors (e.g., lower household income) were uniquely related to specific SIB types. Various medical problems (e.g., headaches) were uniquely linked to SIB types. Greater lethargy and ADHD hyperactive-impulsive symptoms emerged as specific predictors. Seven reactivity items and three dysphoria items were also uniquely associated with specific types.

Each SIB type had a unique set of predictors. For example, 'inserts finger/object' had more medical problem correlates, while 'hits self against surface/object' was linked to more specific emotion dysregulation presentations. Examining correlates that relate to SIB types, with an emphasis on medical problems and emotion dysregulation, may allow for new avenues for SIB interventions for autistic youth.

Poster 114

Understanding Impact of Textural Changes for Mammogram Analysis using Counterfactuals

Arora, Ridhi [1] and Lee, Juhun[1, 2]

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Purpose: This study explored significance of ***texture information*** *(foreground or background)* for lesion detection using ***counterfactuals***, a framework that can explain causal situation by exploring different actions or conditions and its associated results.

Dataset: 10,415 2D screening mammograms with 4,942 recalled lesions with lesion masks and 5,473 normal cases are used. For identifying foreground and background textures, lesion masks extracted from lesion cases, while artificially imposed selected lesion masks on normal controls (***fake*** lesion).

Counterfactual image generation: Examined four counterfactual scenarios:

a) **LF: Gray-out lesion foreground** with mean intensity (MI) vs. **normal** (**N**)
b) **NF: Gray-out normal foreground** with MI vs. **lesion** (**L**)
c) **LB: Gray-out lesion background** with MI vs. **N**
d) **NB: Gray-out normal background** with MI vs. **L**

LF, **NF**, **LB**, **NB**, **L**, **N** have 299 images each, totaling to 598 image in each scenario.

Counterfactual simulation setup: Four test sets (TS-1 to TS-4) with above counterfactuals scenarios were created, with TS-5 having original **L** and **N** images (non-counterfactuals, baseline) for comparison.

Results: Employing CNN models—MobileNet, ResNet50, and ResNet50V2—the study executed ROC and AUC analysis to gauge performance across scenarios. Classifiers on three counterfactual scenarios (**LF vs**. **N** – AUC: 1.00, **NF vs. L** – AUC: 0.98, and **LB vs. N** – AUC: 1.00) performed similar to baseline, while their performances on the cases of **NB vs. L** (AUC: 0.5) were significant dropped (p-value < 0.0001) for MobileNet and improved for ResNet50 (AUC: 0.76) and ResNet50v2 (AUC: 0.92).

Conclusion: The analysis highlights texture modification for mammogram detection, signifying that ***lesion shape*** is ***important*** for classifying lesion images and ***background texture*** is more important for the ***normal image*** classification.

Poster 2

Liver exosomes after hepatic ischemia-reperfusion injury differentially affect bone marrow-derived versus liver-resident dendritic cell function

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Introduction:
Exosomes play pivotal roles in intercellular communication, facilitating delivery of proteins and nucleic acids to proximal and distal cells and are elevated in the circulation following liver ischemia-reperfusion injury (IRI). Dendritic cells (DC) are key regulators of innate and adaptive immunity, here we examined for the first time the influence of exosomes isolated from liver IRI on DC function.

Methods:
C57BL/6 male WT mice underwent 70% hepatic warm ischemia for one hour, followed by six hours reperfusion. Exosomes (<200 nm) were isolated from IRI liver tissue or control liver, purified, and quantified according to the guidelines of the International Society of Extracellular Vesicles. **Bone marrow derived-DC (BMDC) or liver resident-DC were pulsed for 24 hours with the exosomes and phenotypically and functionally analyzed.**

Results:
Liver exosomes were increased significantly following IRI. Their purity was verified by expression of the exosome markers CD63 and CD81, and by absence of the endoplasmic reticulum marker glucose-regulated protein **(GRP)78. Exosomes from ischemic livers (but not those from normal control livers) enhanced the activation and maturation of BMDC, evidenced by increased MHC II and co-stimulatory molecule (CD80/86) expression. This correlated with augmented ability to induce allogeneic CD4 and CD8 T cell proliferative responses in MLR, as well as elevated secretion of proinflammatory cytokines (IFNγ, IL-17A, and TNFα). By contrast, normal liver-resident DCs were refractory to liver IRI-exosome stimulation and** displayed **comparative phenotypic and functional resistance to maturation and acquisition of T cell stimulatory activity.**

Conclusion:
These novel findings reveal a differential capacity of liver interstitial exosomes released following IRI to affect the phenotype and stimulatory function of bone marrow-derived versus liver-resident myeloid DCs. The refractory response of liver-resident DC may contribute to modulation of tissue injury and attenuation of alloimmune reactivity following liver transplantation.

Poster 95

Feasibility of using plasma p-tau217 as a surrogate for brain Aβ PET in the community

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Background: Plasma p-tau217 is probably the best-performing blood biomarker to detect brain amyloid-beta (Aβ) accumulation. However, previous studies were mostly performed in highly selected cohorts. Thus, it is unclear if it can be used independently of confirmatory neuroimaging tests to identify individuals with abnormal brain Aβ load in the wider population. We evaluated the feasibility of plasma p-tau217 replacing Aβ PET in two population-based cohorts.

Methods: This study used data from two cohorts; the Monongahela-Youghiogheny Health Aging Team (MYHAT) Neuroimaging study (n=111, median age 76 IQR: 72-80, 54% female), in a medically and economically-depressed Rust Belt area of southwestern Pennsylvania, and the Human Connectome Project (HCP) (n=229, median age was 62 IQR: 56-70, 65% female) of self-identified Black and non-Hispanic Whites in Pittsburgh city. Plasma p-tau217 was measured using assays from the University of Pittsburgh, ALZpath Inc., and NULISA from Alamar Biosciences. Aβ imaging was performed with ^{11}C-PiB, where global Aβ+ was defined using a threshold of 1.346 SUVR.

Results: The primary analysis used ALZpath p-tau217 for both studies. MYHAT-NI reported a Positive Predictive Value (PPV) of 0.6 and Negative Predictive Value (NPV) of 0.92, as well as sensitivity and specificity of 0.82 and 0.80, respectively. Secondary subset analyses using the UPitt and NULISA p-tau217 assays showed similar performances (PPV: 0.56-0.60, NPV: 0.89-0.93). The primary analysis in HCP showed a PPV of 0.40 and NPV of 0.97, with corresponding sensitivity of 0.85 and specificity of 0.78. The secondary analysis using UPitt p-tau217 performed comparably (PPV: 0.38 and NPV: 0.97).

Conclusion: The consistently high NPV but poor PPV suggest that all the plasma p-tau217 assays will be most useful as a first-screening tool to identify individuals without pathological evidence of brain Aβ for exclusion from clinical trials, therapeutic trials, and prescription of approved therapeutic agents.

Poster 48

Th9/IL-9 as a potential therapeutic target for inflammatory cardiovascular disease

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Psoriasis, a systemic autoimmune disease, is strongly associated with metabolic dysfunction, vascular inflammation, and atherosclerotic cardiovascular disease (CVD). The IL-23/IL-17 pathway is critical for psoriatic inflammation, and targeting these pathways reduces skin, joint, and eye involvement without affecting CVD outcomes. Hence, the potential drivers of psoriatic CVD are still to be elucidated. IL-9-producing CD4+ Th9 cells are found in the circulation of psoriatic patients. We found a significant association of Th9 cells with early radiographic atherosclerotic coronary artery disease in a cohort of patients with psoriasis. We observed an increase in aortic plaque size, and skin/aortic Th9 cells in a psoriatic atherogenesis model (ApoE KO mice treated with IMQ). To understand the involvement of IL-9 as a driver of the disease, we pharmacologically inhibited IL-9 in this model. Mice treated with IL-9 blockade showed decreased skin inflammation compared to isotype-treated mice, which was accompanied by reduced aortic plaque formation. This finding was also corroborated by another novel psoriatic-atherogenesis model devoid of an external psoriasis inducer. In vitro, IL-9 induced endothelial cell dysfunction through STAT3. Together, these results suggest that Th9/IL-9 may be a therapeutic target to prevent atherosclerotic CVD in patients with psoriasis. To further support our finding we are developing in vivo IL-9RKO/ ApoE KO mice model, as well as conditional deletion of IL9R. Further, we plan to study IL-9/STAT3 targets in endothelial cells in vitro.

Poster 115

Exploring the effects of using multiple different cerebellar reference regions to improve tau-PET harmonization - HEAD Study

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Tau-PET tracers have been used to diagnose and stage Alzheimer's disease (AD). However, different tau tracers present distinct patterns of binding throughout the brain, challenging the harmonization of their results. We hypothesize that the choice of a reference region can impact the harmonization of the tau-PET standardized uptake value ratio (SUVR). In this context, we aimed to explore how different cerebellar reference regions impact the association between [^{18}F]Flortaucipir and [^{18}F]MK-6240 SUVR values.

We studied 185 individuals across the aging and AD spectrum with head-to-head Flortaucipir and MK-6240 tau PET (HEAD Study). SUVRs were processed to a common 8mm FWHM using 15 different reference regions defined in the spatially unbiased atlas template of the cerebellum (SUIT) (Diedrichsen, 2009). Regression models investigated the association between Flortaucipir and MK-6240 using multiple combinations of reference regions. R^2 statistic was used to estimate goodness-of-fitting.

We tested 225 combinations of associations Flortaucipir and MK-6240 SUVR, where the SUVRs are not necessarily quantified using the same reference region. Figure 1 presents the top 25 strongest and 25 weakest associations, ordered based on the coefficient of determination (R^2). Flortaucipir and MK-6240 exhibited the most robust associations when the inferior cerebellar gray matter or Crus I were employed for SUVR determination (R^2=0.89, Figure 1-2). Conversely, combinations incorporating the fastigial region consistently yielded weaker associations between the two tracers (R^2<0.70, Figure 1-2).

Interestingly, we showed that the inferior cerebellum, when used for both tracers, serves as a robust reference region for determining the most similar SUVRs for Flortaucipir and MK-6240. Notably, these two regions have been commonly utilized in previous studies involving these tau tracers. Our results imply that the inferior cerebellum could be the optimal reference region for research aimed at harmonizing tau PET tracers using statistical scales.

Poster 49

Treatment with rapamycin increases murine lifespan in a Matrix Gla Protein knockout model of medial arterial calcification.

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Background: Peripheral artery disease (PAD) is characterized by the narrowing and blockage of the vessels in lower extremities. While atherosclerosis has been the assumed culprit of PAD recent data now shows that medial arterial calcification (MAC) is the cause of below-the knee ischemia. MAC promotes inward remodeling, reduced elasticity, and arterial stiffness. The mechanisms behind MAC remain unclear. Matrix Gla Protein (MGP) is an inhibitor of vascular calcification and present in arterial vessel walls. We observed that $MGP^{-/-}$ mice died within 5-7 weeks due to aortic rupture or heart failure. The drug rapamycin functions as an inhibitor of cell proliferation and an activator of autophagy.

Hypothesis: That rapamycin reduces MAC in $MGP^{-/-}$ mice via inhibiting inward remodeling and activating autophagy.

Methods: $MGP^{+/+}$ and $MGP^{-/-}$ mice treated with vehicle or rapamycin (5mg/kg, 3x weekly) starting at 10 days old for 2 weeks. Calcification was quantified via micro-computed tomography (uCT). Collagen structure and elastin quality were assessed histological staining. Markers of proliferation (Ki-67), cell death (Caspase 3), autophagy (LC3), and calcification (Runx2) were quantified via immunofluorescence staining.

Results: We observed $MGP^{-/-}$mice treated with rapamycin lived longer than the vehicle-treated mice. The content of calcification was the same between $MGP^{-/-}$ mice compared to the vehicle, but a significant decline in mineral density was observed in $MGP^{-/-}$ mice treated with rapamycin. Higher collagen content and improved elastin structure were observed in MGP-/- treated with rapamycin compared to vehicle. Runx2 and Ki-67 were elevated in $MGP^{-/-}$ mice compared to $MGP^{+/+}$, but there was no difference in response to rapamycin treatment. No difference was observed in caspase 3 levels.

Conclusion: Our data suggest that rapamycin potentially preserves the integrity of the aortic vessel wall through a mechanism other than autophagy or proliferation, indicating the need for further investigation.

Poster 3

Novel Ligand-Inducible TDP-43 (LIT-43) System to Studying Key ALS Disease Mechanisms

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TDP-43 is a predominantly nuclear RNA-binding protein found mislocalized and forming insoluble cytoplasmatic aggregated within neurons of Amyotrophic lateral sclerosis (ALS) patients. In normal physiology, TDP-43 resides in the nucleus within functional biomolecular condensates regulated by phase separation of proteins and nucleic acids. These TDP-43 condensates allow for spatiotemporal control of localized functions, including the regulation of splicing, nuclear export, trafficking in the cytoplasm, translation, and degradation. Changes in biomolecular condensate dynamics are hypothesized to play a key role in neurodegenerative diseases, including ALS. However, the cellular and molecular mechanisms leading to TDP-43 mislocalization, aberrant condensation, and loss-of-RNA-binding are poorly understood. Unfortunately, to answer these outstanding questions, few relevant models exist to study the cellular mechanisms driving aberrant TDP-43 phase transitions. Here, we introduced a Ligand-Inducible TDP-43 (LIT-43) system, a novel cellular model to understand the hallmark events occurring in ALS. By integrating the broad-complex, tram-track, and bric-a-brac (BTB) domain at the N-terminal of TDP-43, the BTB region functions as a ligand-inducible oligomerization switch, allowing selective induction and reversal TDP-43 mislocalization and aggregation in human cells. Additionally, we establish a direct link between the aggregation achieved in our model and a loss of TDP-43 splicing function and autoregulation, thereby enabling the study of the key pathological features observed in ALS.

Poster 4

Optimizing Fc-engineered novel anti-MSLN VH-Fc fusion proteins through PET imaging

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Introduction: The tumor associated protein mesothelin (MSLN) is overexpressed in numerous cancers, making it a viable target for therapies. Recently, we developed and evaluated [^{89}Zr]Zr-2A10-VH-Fc, anti-MSLN VH-Fc fusion protein, and compared it to the M912-IgG1 through [^{89}Zr]Zr-labeled PET/CT imaging in murine model of human colorectal cancer (CRC) using HCT116 cells. The preliminary investigation of the [^{89}Zr]Zr-2A10-VH-Fc fusion proteins resulted high tumor accumulation and penetration as compared to the [^{89}Zr]Zr-anti-MSLN IgG1; however, accumulation in the spleen and liver was observed due to binding to Fcγ receptors. To address this non-MSLN binding, we aim to compare the pharmacokinetics profiles of VH-Fc mutants that do not bind Fcγ receptors as compared to the wild-type (2A10-VH-Fc$_{WT}$). The overall objective is to develop optimal anti-MSLN VH-Fc fusion proteins as targeting vectors for delivering imaging or potentially therapeutic payloads to MSLN-expressing tumors.
Methods: Mutant 2A10-VH-Fcs were engineered (Fc$_{GRLR}$ and Fc$_{LALAPG}$), modified to present the DFO chelator for radiolabeling with Zr-89. *In vivo* stability studies were conducted in normal mice at 1-day post-injection (p.i.). PET/CT imaging was performed in NCG mice bearing HCT116 tumors at 90 minutes; 1, 2, and 5-days p.i. and a biodistribution study was performed following the 5-day imaging timepoint. Microscale distribution was evaluated by iQID imaging at 1-day p.i.
Results: DFO-modified proteins were successfully radiolabeled with Zr-89 in high yields (>95%). [^{89}Zr]Zr-labeled VH-Fc mutants exhibited high in vivo stability (>90%) as compared to WT at 1-day p.i. PET/CT imaging revealed increased tumor accumulation and retention for the both [^{89}Zr]Zr-2A10-VH-Fc$_{LALAPG}$ and [^{89}Zr]Zr-2A10-VH-Fc$_{GRLR}$ as compared to the [^{89}Zr]Zr-2A10-VH-Fc$_{WT}$. At 120 hours p.i., the VH-Fc mutants resulted substantial reduction in spleen and liver uptake. The mutants exhibited increased kidney uptake, indicating greater kidney clearance than the WT. *Ex vivo* biodistributions demonstrated increased tumor uptake of [^{89}Zr]Zr-2A10-VH-Fc$_{LALAPG}$ and -VH-Fc$_{GRLR}$ (13.7 ± 0.6% ID/g, 10.5 ± 1.2%ID/g, respectively) as compared to [^{89}Zr]Zr-2A10-VH-Fc$_{WT}$ (4.2 ± 0.5% ID/g). iQID images findings were in line with PET/CT and biodistribution studies.
Conclusion: In summary, we successfully generated two Fc-null anti-MSLN VH-Fc fusion proteins and affirmed their suitability for delivering a payload to MSLN-positive tumors without being impacted by non-MSLN binding. PET/CT imaging demonstrated the mutants had improved pharmacokinetic profiles (enhanced tumor uptake while reducing spleen and liver uptake) as compared to the WT. Biodistribution and iQID imaging supported the PET/CT findings. Overall, these data are encouraging and support the continued development of anti-MSLN fusion proteins as imaging and potentially therapeutic agents.

Poster 5

Title: cGAS Deficiency is Associated with Altered Tumor Microenvironment (TME) and Dysfunctional Autophagy Leading to Increased Colitis associated Colorectal Cancers (CAC).

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CACs are a critical concern tied to IBD. Previously, we showed that cGAS is upregulated in human UC and murine colitis, and cGAS deficiency leads to worse intestinal inflammation. Our current research is on the role of cGAS in CAC. Our hypothesis is cGAS deficiency alters the TME and autophagy leading to increased tumorigenesis.

We subjected WT, cGAS KO, Villincre (VC) cGASfl/fl, & Lysmcre (LC) cGASfl/fl mice to the AOM/DSS model. Tumor counts, H&E staining, WB analysis, and flow cytometry of the tumors were performed. We also subjected VC; cGASfl/fl mice to rapamycin, an inducer of autophagy.

Our data shows an increase in cGAS protein levels in the tumors of WT mice subjected to AOM/DSS compared control & DSS colitis colonic tissue *($n=8$, $p<0.05$).* When cGAS KO mice were subjected to AOM/DSS, they had significantly more colonic tumors compared to WT mice (*$n=8$, $p<0.001$*). H&E- sections showed no significant difference in colonic inflammation (*$n=6$, $p=0.82$*), but significantly higher dysplasia in the colonic tumors of cGAS KO mice (*$n=6$, $p=0.007$*). Analysis of the immune cell populations of the murine TME via flow cytometry show a decrease in macrophages, an increase in MDSCs, decreased activated CD4+ and CD8+ T cells, and altered transcription factors: FoxP3+, Tbet+, and RORγt+ cells showing an immunosuppressed TME. LC cGASfl/fl mice had significantly more tumors compared to their floxed controls with a trend towards increased dysplasia in these tumors. When we subjected VC cGASfl/fl mice to AOM/DSS, they showed higher number of colonic tumors with increased dysplasia despite no difference in inflammation scores (*$n=8$, $p<0.02$*). WB analysis showed decreased LC3-II and SQSTM1/p-62, indicating autophagic dysfunction (*$n=8$, $p<0.05$*). When subjected to rapamycin, we see a trend towards decreased tumors in the VC cGASfl/fl.

cGAS deficiency is associated with increased tumorigenesis possibly due to an immunosuppressed TME and epithelial autophagy. Collectively, these results suggest that enhancing cGAS activity or autophagy by an inducer could be promising strategies for CAC prevention and treatment.

Poster 50

Novel reparative regulatory T cell therapy promotes repair and transplant survival when delivered early after transplantation

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Introduction: Tissue damage leads to the release of typically sequestered molecules that alert the immune system to the injury. IL-33 is released from the nucleus during injury to stimulate cells expressing the IL-33R, "ST2". Mouse (mu) models identified a beneficial role for IL-33 in tissue repair partially due to stimulating $ST2^+$ T regulatory cells (Tregs). Treg repair mechanisms are independent of their suppressive functions and involve the secretion of the growth factor Amphiregulin and IL-13. Here, we describe seminal attempts to expand and harness reparative human (hu) and mu IL-33-stimulated Tregs as a novel cell therapy in transplantation.

Methods: Spleen mu $CD4^+$ $CD25^{hi}$ $CD127^{lo}$ $FoxP3\text{-}RFP^+$ Tregs and CD4+ CD25HI CD127lo Tregs isolated from healthy hu donors were expanded with expansion beads or L-cell based APCs respectively and IL-2 +/- IL-33 for 21 days. Phenotype and function were assessed using flow cytometry and *in vitro* assays. In an MHC-II mismatch skin transplantation model, B6 mice received Bm12 skin graft and IL-2 or IL-33 expanded Tregs and were monitored for graft survival. Changes in systemic and local immunobiology were assessed in a second cohort on day 10.

Results: IL-2 and IL-33-stimulated Tregs expanded in a comparable fashion. IL-33 stimulated Tregs displayed upregulation of ST2, CCR4, CTLA-4, PD-1 and IL-13. Where both types of Treg had comparable suppressive capacity, IL-33-stimulated Tregs outperformed IL-2 expanded ones in accelerating wound closure. Treg cell therapy could promote allograft survival in the absence of immunosuppression, however, IL-33-stimulated Treg increased reparative/regulatory macrophages locally and up-regulated repair pathways relative to IL-2-ones.

Conclusions: We show that IL-33 can be used in mu and hu Treg expansion cultures to generate suppressive and reparative Tregs that upregulate ST2, CCR4, CTLA4, PD-1, and produce IL-13. Importantly, IL-33-stimulated Tregs exhibit augmented reparative functions in vitro and were highly effective at promoting allograft survival when delivered after transplantation.

Poster 51

Biological Changes in Ameloblasts of AmelxSer16Ala Knock-in Mice

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The major extracellular enamel protein amelogenin (AMELX) has a single phosphorylation site at Ser16. To investigate the role of AMELX phosphorylation in amelogenesis, we have established a knock-in (KI) mouse in which Ser16 was substituted with Ala (AMELXS16A KI). This substitution led to the increase in the rate of amorphous calcium phosphate (ACP) to apatite transformation, major changes in enamel structure and mineral composition, and to ameloblast cell pathology. We hypothesized that the increased rate of mineralization can lead to enamel acidification, which can affect matrix assembly and ameloblast biology. Specifically, we hypothesized that these changes might affect pH homeostasis machinery and ameloblasts differentiation.

Strips of developing enamel organs from incisors were dissected into five segments corresponding to secretory, mixed secretory/transition/early maturation, mid-maturation, and late maturation, using a novel method developed by us [2]. Expression of enamel matrix proteins (EMPs) from secretory and maturation stages of amelogenesis and key pH regulators at the gene and protein levels in ameloblasts of KI and wild-type (WT) mice was assessed using RT-PCR, Western Blot and immunohistochemistry. Micro-CT and TEM were used to assess structural differences between KI and WT.

Compared to WT mice, in KI enamel, prolonged expression of secretory EMPs (Amelx, Enam) and lower expression of maturation EMPs (Amtn, Odam) suggests a histodifferentiation delay. There were significant changes in expression of carbonic anhydrases (CAs) family, major pH regulators, during secretory stage. We also observed delay in the onset of enamel deposition and defects of basal lamina during the maturation stage in KI. Together results corroborate the crucial role of Amelx phosphorylation in the cellular function and differentiation of ameloblasts.

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Poster 34

Conceptualizing Food Insecurity During Pregnancy Through Community-Engaged, Mixed-Methods Research: Disruptions to Food Access and Associated Distress

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Food insecurity (limited or uncertain access to sufficient food) affects 20% of U.S. pregnant individuals and is linked to adverse prenatal and infant health. Extant conceptualizations of food insecurity do not account for relevant prenatal experiences (e.g., changes in nutritional needs), limiting the accurate identification of and support for pregnant individuals with food insecurity. This mixed-methods, community-engaged study aimed to explore how pregnancy uniquely affects food insecurity and identify the core features of prenatal food insecurity.

Participants ($N = 43$; 91% Black-identifying; 30.3±3.7 years old; 14.2±10.0 weeks gestation) were pregnant individuals living in the U.S. who endorsed food insecurity on the two-item Hunger Vital Signs measure. A PhD-level researcher facilitated four iterative 90-minute focus groups. Participants also completed validated measures of food insecurity and related constructs. Focus group transcripts were analyzed using framework analysis supplemented by survey data to enhance data triangulation and sample characterization.

Pregnancy altered the experience of food insecurity by posing unique challenges to food access (e.g., fatigue limits ability to walk to affordable stores), food rationing (e.g., due to increased food cravings), and psychosocial distress (e.g., worry about developing baby's nutrition). The core features of prenatal food insecurity fell under two primary themes: disruptions to food access and associated distress. Three subthemes emerged under disruptions to food access: insufficient food quantity, nutrition quality, and preferred foods. Four subthemes emerged under distress: psychological (e.g., anxiety), physiological (e.g., food cravings), social (e.g., stigma), and cognitive (e.g., significant planning for food access).

Findings underscore the multidimensional impact of prenatal food insecurity. Understanding these nuances is essential for addressing the complex interplay between food insecurity and perinatal health to foster holistic care for pregnant individuals.

Poster 6

X-ray crystallographic studies of the ancestral steroidogenic cytochrome P450 11A1 enzyme

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The ancestral cytochrome P450 11A1 (CYP11A1) enzyme is responsible for the metabolism of steroid hormones. The isoform CYP11A1 catalyzes the cleavage of side-chain cholesterol into pregnenolone. The latter is the precursor of steroid hormones such as glucocorticoids, mineralocorticoids, and sex hormones. The CYP11A1 vertebrate ancestor was previously reconstructed and biochemically characterized and presented a high thermostability and differences in substrate specificity compared to the bovine isoform. We characterize the threedimensional structure of the vertebrate ancestor by X-ray crystallography to better understand the structure-function relationship of the CYP11A1 isoform and its altered substrate specificity. The recombinant CYP11A1 protein was expressed and purified in a monodisperse state in solution. Crystallization screens were proceeded and a condition promoting crystal growth identified. Crystals were further optimized and resulted in a cubic-shaped crystal. The diffraction data was collected at the Stanford Synchrotron Light Source (SSRL). The structure was processed to atomic resolution at 2.4 Å in a high symmetry space group. Preliminary data indicates modifications of the substrate access channel and recognition site compared to the human and bovine CYP11A1 isoforms. The structure of CYP11A1 vertebrate will contribute to further knowledge of substrate preference in the different isoforms and shed light on the evolution of human steroidogenesis.

Hartz, P.; Strohmaier, S.J.; El-Gayar, B.M.; Abdulmughni, A.; Hutter, M.C.; Hannemann, Gillam, E.M.J.; Bernhardt, R. Resurrection and characterization of ancestral CYP11A1 enzymes, FEBS J. 2021 Nov;288(22):6510-6527

Poster 96

Sleep, Hypertension, and Nocturia: Multicomponent Approach for Comorbid Illnesses

Chahal, Rutvi;[1] Newell, Kandy; [1] Zhang, Gehui; [2] Zhao, Yichu; [1] Dawson, Jordan; [1] Perera, Subashan; [1] Clarkson, Becky; [1] Tyagi, Shachi; [1]

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Frequent sleep interruptions and nocturia are known to increase nighttime systolic blood pressure (SBP) which results in poor awake hypertensive (HTN) control, nighttime nondipping and adverse cardiovascular outcomes. Effects of behavioral sleep intervention (BBTI) or chronotherapy (CHR) on nighttime nondipping or awake BP control in older adults are unclear. Current study is a pilot randomized controlled trial to assess efficacy and tolerability of these interventions.

We randomized 30 community-dwelling adults (mean age, 72±5 yr; 57% women) on ≥1 daily non-diuretic anti-HTNs and awaken ≥2 times nightly to void to one of 3 groups **i) control:** continue morning anti-HTN dosing, **ii) BBTI** and continue morning anti-HTN, or **iii) CHR:** switch to bedtime anti-HTN dosing for 6-weeks. All participants completed three-day bladder diary to assess nocturia frequency, nighttime urine volume (NUV), and nocturnal polyuria index (NPi: percent of 24h urine voided during sleep). Ambulatory monitor was used to record 24h BP. In home sleep efficiency was calculated with the concurrently worn single-channel, EEG device (Z-machine®).

BBTI arm showed a decline in nocturia frequency (-0.6±0.7, p=0.03) and NPi (-6.6±3.2, p=0.05). Decreased nocturia frequency correlated with decline in awake (r=0.69, p=0.03) and asleep SBP (r=0.59, p=0.07). Reduced NPi correlated with nighttime dipping in SBP (r=0.75, p=0.01). Increase in sleep efficiency in BBTI group correlated with increased nighttime dipping (r=0.62, p=0.05) and decline in awake (r=-0.78, p<0.01) and asleep (r=-0.82, p<0.01) SBP. These bladder or sleep parameters remained unchanged in the CHR group. Both BBTI (p=0.04) and CHR (p<0.01) groups demonstrated a significant nighttime dipping of SBP post-intervention. All groups demonstrated tolerability (no lightheadedness or falls) to the interventions.

BBTI and CHR were well tolerated and demonstrated decreased nighttime SBP. Additionally, BBTI improved sleep, nocturia, awake SBP and nocturnal dipping. Future work will focus on validating these findings in larger datasets.

Poster 97

The relationship between Urinary Urge Incontinence and gait in older women

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Urgency urinary incontinence (UUI) a prevalent, morbid condition in older adults significantly affects patients' quality of life. Falls affect 1 in 4 older adults. Factors leading to falls remain understudied. This study aims to analyze the impact of UUI on gait measures, specifically gait speed and sit-to-stand (STS) time, which are previously proven markers for fall risk.

A retrospective analysis was conducted using gait test data in continent controls without UUI and incontinent patients with UUI. Variables, including age, weight, height, BMI, mean leaks from 3-day bladder diaries, and gait speed test, 5 STS time, were collected. Descriptive statistics, two-sample t-tests, and linear regression for the dependent variables including gait speed and STS time were performed using R.

Out of 52 participants, 18 did not have UUI and leakage, while 34 had UUI. The non-UUI control group had mean age 64±4.9 yrs., BMI 26.9±4.6 kg/m^2, leak episodes 0±0.1, gait speed 1.1±0.5 m/s, and STS time of 11.5±2.7 s. The UUI group showed mean age 69±7.4 yrs., BMI 31.7±6.5 kg/m^2, leaks 0±3.4, gait speed 0.6±0.4 m/s, and STS time of 13.7±5.6 s. The t-test demonstrated mean gait speed for the UUI group was significantly slower than controls **(p=0.00).** Linear regression for gait speed revealed that age, BMI, and leak episodes significantly predicted gait speed $F(3, 48)=5.147$, **p=0.003**, $R^2=0.24$, with BMI $\beta=-0.02$, $t=-2.05$, **p=0.04** and leaks $\beta=-0.05$, $t=-2.17$, **p=0.03** having a significant negative impact on mean gait speed. Regression for STS time revealed that age, BMI, and leak episodes significantly predicted STS time $F(3, 45)=3.22$, **p=0.031**, $R^2=0.17$, with BMI $\beta=0.2$, $t=2.5$, **p=0.01** having a significant positive impact on mean STS time.

This study establishes a negative impact of leaks and BMI on gait speed. We did not find associations of STS time with leaks but revealed a significant influence of BMI on it. Although only a limited amount of variability in gait speed and STS is explainable by regression, it underscores the significance of conducting additional gait analyses for exploring the factors contributing to falls.

Poster 116

Genetically encoded gold nanoparticles for anti-cancer photothermal therapy

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Despite having multiple advantages as instruments for drug delivery in cancer therapy, metal nanoparticles (NPs) have shortcomings that make them less applicable as anti-neoplastic agents. One major shortcoming is the very small fraction (~0.002%) of the NPs reaches the intended location in the cell or tissue. This is due to loss of NPs in the blood, interstitial fluid, or off-target cells. Here, we circumvent this obstacle by having the target cells genetically encode the intracellular synthesis of gold NPs. The gold NPs are produced by biomineralization of metal ions by exogenous peptides. This novel NP synthesis process allows the precise control over the size, number, geometry, and intracellular distribution of the NPs. As a proof of concept, we synthesized the gold NPs in glioblastoma cells and used them for targeted ablation of tumors through laser induced photothermal therapy. This process utilizes the effects of surface plasmon resonance, a unique property of gold NPs by which they convert incident near infra-red radiation to localized heat which disrupts cellular processes. This platform can be generally utilized for designing other techniques and imaging modalities for cancer theranostics.

Poster 52

Functional characterization of the novel cell adhesion molecule MPZL3 in ovarian cancer

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Ovarian cancer (OVCA) is the most lethal gynecological malignancy in the United States often detected at advanced stages. OVCA progression involves transcoelomic metastasis, where cells disseminate into the peritoneal fluid, adhere to form multicellular aggregates that promote anchorage-independent survival and facilitate metastatic colonization of the peritoneum. To meet the requirements of each stage of this detachment, aggregation, and re-attachment cycle, OVCA cells have been shown to dynamically regulate the expression of multiple cell adhesion molecules (CAMs) throughout their progression. Moreover, targeting cell-cell adhesion molecules has shown to be an effective method to slow/inhibit the progression of OVCA.

Myelin protein zero-like 3 (MPZL3) is a transmembrane protein with homology to other immunoglobulin-like (Ig) family of CAMs. While it has been reported that altered Ig-CAM expression plays a role in ovarian cancer, the function of MPZL3 has not been investigated. TCGA data shows frequent chromosomal loss of the *MPZL3* locus (11q23.3) in various cancers, including high grade serous ovarian cancers (HGSOC), suggesting that loss of genes located in this area has tumorigenic consequences.

To study the function of MPZL3 in OVCA, we used shRNA mediated MPZL3 knockdown in OVCAR4 human HGSOC cells and examined transcriptome-wide effects by RNA sequencing. We found that loss of MPZL3 resulted in decreased cell growth with a concomitant resistance to both Cisplatin and Olaparib treatments, both of which are commonly used for treating OVCA. Moreover, we demonstrated that knockdown of MPZL3 decreased the homotypic adhesive capacity and promoted invasiveness of OVCA cells. Ongoing studies are exploring the mechanistic link between MPZL3 loss and chemoresistance and determining the effects on in vivo tumor progression. Understanding the novel role of MPZL3 will provide further insight into OVCA progression, thus generating opportunities of developing new treatments for patients with low MPZL3 expression as an approach for precision cancer medicine.

Poster 7

Macrophage-specific VCAM1 contributes to pathogenesis during influenza-induced exacerbation of atherosclerosis.

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Influenza is a significant public health and economic threat around the world. Although pneumonia is the most common complication associated with influenza, there are several clinical reports demonstrating increased risk for cardiovascular disease. Studies have shown that influenza infection correlates with increased incidence of myocardial infarction. Atherosclerosis is the most known cause of ischemic heart diseases and stroke. Vascular cell adhesion molecule-1 (VCAM1) has been shown to promote adhesion of monocytes and promotes atherosclerosis. In this study, we hypothesize that VCAM1 plays a role in exacerbation of atherosclerosis during influenza infection. WT and $ApoE^{-/-}$ were fed with a high-fat diet (HFD) for 11 weeks. Mice were then infected with influenza A/PR/8/34 (H1N1) or PBS (vehicle control), and weight loss, survival, and gene expression of vascular endothelial adhesion molecules, inflammatory cytokines and chemokines were measured. HFD-fed $ApoE^{-/-}$ mice were infected with influenza, and treated with anti-VCAM1 antibody, and HFD-fed $ApoE^{-/-}VCAM1^{fl}LyzM^{Cre}$ mice were treated with influenza, and weight loss and survival were measured. Increased weight loss and decreased survival of mice were observed in response to influenza infection in HFD-induced atherosclerosis in $ApoE^{-/-}$ mice. Further, the expression of VCAM1, and the levels of IL-6, CCL2, CCL3, CCL5 were significantly increased in aorta in $ApoE^{-/-}$ mice when compared to PBS-treated controls. Increased survival and decreased weight loss in response to antibody-mediated VCAM1 neutralization or macrophage-specific VCAM1 deletion during influenza infection was observed. These results suggest that the expression of VCAM1 on macrophages plays a role in the influenza-induced exacerbation of atherosclerosis.

Poster 117

A 3D Bioprinted Proximal Tubule Model for the Investigation of Hypokalemia Induced Ammoniagenesis

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Ammoniagenesis, the generation of ammonium from glutamine, is a key mechanism in the kidney for excretion of acid and conservation of potassium ions [K+]. While hypokalemia (low [K+]) is associated with upregulated ammoniagenesis, the underlying mechanism is poorly understood and challenging to study in vitro due to the limitations of current kidney proximal tubule (PT) models. Permeable supports utilized to study kidney physiology lack tubule-like structural constraints, luminal flow, or a 3D microenvironment, required to induce and maintain cellular maturity. In this study, we developed an innovative proximal tubule model that combines traditional orbital shakers used in permeable support studies with 3D bioprinted collagen-I circumferential inserts.

Freeform reversible embedding of suspended hydrogels 3D bioprinting was employed to fabricate microporous flat and channeled 3D collagen scaffolds and inspected for dimensional accuracy using optical coherence tomography. Printed scaffolds were placed into Transwell inserts and seeded with opossum kidney PT (OK) cells to assess the growth and transport compared to a conventional 2D Transwell model. Tissues were cultured for statically for 4 days then matured by rotational shear for an additional 4, 7 and 10 days. OK cellular functionality and viability was assessed by albumin uptake and calcien-AM and EthD-1 fluorescent staining respectively. Interestingly, the OK cells not only formed a monolayer along the channel surface but also displayed evidence of ECM remodeling and growth into the bulk of the 3D bioprinted collagen.

In conclusion, this 3D bioprinted microfluidic proximal tubule model will facilitate studying kidney cells in a more physiologic environment to identify the key mechanisms controlling ammoniagenesis. Our initial data suggests that collagen-I is sufficient for OK cell adhesion and proliferation; however, by changing the ECM composition and architecture, we can vary our models mechanical properties, adhesion molecules, growth factors, and other elements that are essential to optimize this PT model.

Poster 8

The Non-Canonical Role of TERT in Cardiovascular Calcification

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Cardiovascular calcification is a complex disease with high prevalence in the aging population. The osteogenic reprogramming of vascular cells is central to this disease. Calcific aortic valve disease (CAVD) is the leading heart valve disorder and resembles osteoblast osteogenesis in skeletal bone formation. The precise mechanism by which a vascular cell transforms into an osteogenic state remains to be determined. Telomerase is a protein-RNA complex best known for maintaining telomere length. TERT exerts transcriptional and chromatin remodeling activities independent of its canonical telomerase activity. We show evidence that TERT is highly expressed in CAVD valves, that genetic depletion of TERT reduces calcification of human primary aortic valve interstitial cells (hVICs), and that TERT binds the Signal Transducer and Activator of Transcription 5 (STAT5) to promote the expression of RUNX2, a key transcription factor involved in osteogenic differentiation, independent of its canonical telomerase activity. Our findings were validated in *Tert*-knockout mice, where *Tert* deficiency impairs calcification in vivo and ex vivo. Preliminary evidence shows that genes exhibiting STAT5 occupancy are associated with chromatin remodeling and osteogenesis. During osteogenesis, we found that TERT and STAT5 interact with SMARCA4, a core subunit of the SWI/SNF chromatin remodeler complex. Our findings show that the non-canonicalTERT transcriptional regulatory functions are operative in calculating hVICs and shed light on the early step that regulates the osteogenic transdifferentiation of hVICs.

Poster 98

Racial Disparities in Birth and Lactation Outcomes Among Neonates with Critical Congenital Heart Defects

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Background: Despite evidence of racial disparities in birth and lactation outcomes, the ways in which systemic racism impacts birth and lactation among neonates with congenital heart defects (CHD) is unclear. Thus, we sought to discern if racial disparities exist in birth and lactation outcomes among a cohort of neonates with CHD by exploring associations with individual and community-level indicators for systemic racism.

Methods: This was a *post hoc* analysis of retrospective electronic health record (EHR) data from neonates with CHD (n=20) hospitalized at a mid-Atlantic children's hospital between April 1st, 2016 and April 30th, 2020. Systemic racism was operationalized as neonates' EHR documented race and Conduent Healthy Communities Institute's Food Insecurity Index and Health Equity Index scores, derived from the neonates' EHR documented 5-digit zip codes. Primary outcomes were birth weight, birth weight-for-age z-score, and percentage of enteral feeds that were parental milk and human milk in the first 28 days of life. Regression (linear, logistic, mixed level) were applied for analysis.

Results: Higher Food Insecurity Index score was associated with lower birth weight (p = .014), weight-for age z-scores (p = .026), and lower human milk percentage (p = .003). Neonates documented as being "Black" or "Black and White" had lower parental milk (p = .003) and human milk (p <.001) percentage. Higher Health Equity Index score was associated with lower human milk percentage (p = .005).

Conclusions: Significant racial inequities in birth and lactation outcomes exist for this cohort of neonates with CHD. More research is needed to corroborate these findings.

Poster 9

Phosphoproteomic characterization of the dorsal anterior cingulate cortex (dACC) in Schizophrenia

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Schizophrenia is a polygenic psychiatric disease characterized by impairments in sensory processing, social interactions, and cognition, as well as hallucinations and delusions. Anatomical and physiological studies implicate the anterior cingulate cortex (ACC) as one of the regions contributing to schizophrenia symptoms. The ACC comprises four regions, including the dorsal anterior cingulate cortex (dACC), which connects major brain systems associated with emotion, cognition, and executive function. Determining the underlying molecular regulatory physiology in these patients is critical to understanding schizophrenia's etiology. Unbiased genetic studies implicate multiple kinases and phosphatases in schizophrenia. Phosphorylation is a common regulatory post-translational modification modulating protein function. Identifying dysregulated phosphorylation within the dACC of schizophrenia patients associated with kinases and signaling proteins will provide insight into the mechanistic neurobiology of schizophrenia. Postmortem dACC grey matter was dissected from 56 schizophrenia subjects, each paired with an unaffected control, matched for sex, age, and postmortem interval (PMI). The tissue was homogenized with a bullet blender, and proteins were solubilized and digested using S-TRAP(Protif). Peptide digests were randomized into 8 groups of 14 samples with 2 controls and labeled with TMTpro. Phosphopeptide enrichment was performed with AssayMAP (Agilent), fractionated with high pH reverse phase chromatography. Data were analyzed with Proteome Discoverer (2.5) and Perseus (2.0.11). We quantified 27,607 phosphopeptides corresponding to 5,370 proteins ($q<0.05$). Of these, 174 phosphopeptides on 128 proteins were upregulated ($q<0.05$, fold change (FC) >1.5), while only 7 phosphopeptides corresponding to 6 proteins ($FC<0.67$, $q<0.05$) were downregulated. This experiment is the deepest and most well-powered characterization of the phosphoproteome in any brain region in any psychiatric disease.

Poster 10

Distinct roles of IL-17- induced transcription factor C/EBPδ in kidney inflammatory disease

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Renal tissues are susceptible to inflammation and autoimmune reactions. The incidence of chronic kidney disease associated mortality is alarming, but our understanding of the underlying disease pathogenesis remains poorly understood. IL-17 is a key driver of renal inflammation and mediates signals through multiple transcription factors (TFs) including members of the NF-κB, AP-1 and CCAAT/Enhancer binding protein (C/EBP) families. C/EBPδ was identified over 30 years ago, yet its contributions to immune function are not well understood. IL-17 rapidly and potently induces C/EBPδ in target cells, including renal epithelial cells, and regulates genes associated with kidney disease such as lipocalin-2 (Lcn2, also known as NGAL or 24p3). This study depicts the contrasting role of C/EBPδ in kidney disease. Here we show that C/EBPδ is markedly enhanced in the kidney of patients with autoantibody-induced glomerulonephritis (AGN) as well as in the corresponding mouse model of AGN. *Cebpd*$^{-/-}$ mice were refractory to inflammation, with no changes in markers of renal injury such as blood urea nitrogen (BUN) and creatinine. Expression of C/EBPδ in non-hematopoietic cells was required to drive disease pathology. C/EBPδ binds to the promoter of kidney injury marker genes in mouse and human renal epithelial cells, including *IL6* and *LCN2*. However, neither IL-17 nor C/EBPδ were required to drive kidney fibrotic disease triggered by the nephrotoxin aristolochic acid I (AAI). Surprisingly, upon AAI administration, *Cebpd*$^{-/-}$ mice displayed increased blood urea nitrogen, renal collagen deposition, and decreased expressions of ECM-degradation genes, indicative of a protective role of C/EBPδ in renal fibrotic damage.

Poster 118

Enhancing LoRaWAN for Real-Time IoT Applications through Advanced Scheduling Algorithms

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LoRaWAN has emerged as a pivotal Low-Power Wide-Area Network (LPWAN) standard for Internet of Things (IoT) applications due to its long-range communication capabilities, low-power requirements, and license-free ISM band usage. These features significantly reduce deployment and operational costs, making it an attractive option for private network deployments. However, for industrial adoption, LoRaWAN must address its scalability and reliability limitations, particularly in real-time data transmission. This research introduces a novel real-time, collision-free scheduling algorithm fully compatible with existing LoRaWAN standards. The algorithm leverages graph coloring techniques to efficiently schedule transmissions, thereby minimizing packet collisions – a common issue due to the Aloha-based protocol used by LoRaWAN. Our evaluation, conducted through NS-3 simulations, demonstrates the algorithm's effectiveness in drastically reducing packet loss rates to near zero under various network configurations, including multiple gateways and devices. This enhancement enables reliable and timely data transmission, a critical requirement for real-time applications in IoT deployments.

Poster 53

Discovery of a new antiretroviral drug candidate targeting the RNAse H activity of HIV reverse transcriptase

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Thirty-nine million people worldwide are infected with HIV. While effective treatments have been developed, acquired drug resistance poses a threat towards long term effectiveness. The enzyme reverse transcriptase (RT) is responsible for converting viral ssRNA into dsDNA: a crucial step for viral replication. Due to the importance of this step, RT has made for an effective target for antiretroviral development. The protein requires both polymerase and RNAse H activity to be effective. To date, commercial drugs have only targeted the former. Our aim was to identify potential inhibitors for the RNAse H ability of RT.

We used a high-throughput screen to identify potential inhibitors of RT, and representatives for the most successful hits. These compounds were further tested for inhibition and cytotoxicity, and we then selected commercially available compounds similar to the most promising inhibitors. These were all tested for their ability to inhibit both the polymerase and RNAse H functions.

We found four successful candidates. Compounds **1-3** has IC50s in the high nanomolar range, while **4** was in the low micromolar range. Compounds **1-3** also inhibited polymerase activity, although less effectively, with the IC50s in the low micromolar range. In contrast, compound **4** did not inhibit RT's polymerase activity. All four compounds exhibited activity against common drug resistant mutants. We have successfully identified four potential HIV RT RNAse H inhibitors, one of which is specific only to the RNAse H activity of RT. These are a promising class of inhibitors, which could provide an exciting new target for antiretroviral drugs.

Poster 35

Exploring the Link Between Emotional Reactivity and Early Childhood Aggression in Autistic Preschoolers: A Machine Learning Clustering Perspective

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Despite heightened rates of aggressive behaviors among older autistic youth relative to non-autistic peers, less is known about these behaviors during early childhood. This study aims to identify distinct subgroups of autistic preschoolers based on their presentations of aggression, emotional reactivity, and autism traits. Data were analyzed from parents of 622 (2- to 5) year-olds autistic children who completed the Multidimensional Assessment Profile of Disruptive Behavior (MAP-DB)-Aggression subscale and the Emotion Dysregulation Inventory-Young Child (EDI-YC)-Reactivity subscale. A random forests machine learning clustering technique was applied to the item level data of the EDI-YC Reactivity, MAP-DB Aggression, and social communication questionnaire (SCQ) items, revealing three distinct subgroups of autistic preschoolers. Group 1 exhibited low levels of both reactivity and aggression, group 3 exhibited high levels of both, and group 2 displayed relatively high reactivity but low levels of aggression. Notably, speaking ability and intellectual disability did not differ significantly among the subgroups, but subgroup 3 had more children with diagnosed or suspected ADHD. The most influential factors for clustering decisions were specific aggression items from the MAP-DB and reactivity items from the EDI-YC. SCQ items, on the other hand, showed minimal contribution towards clustering membership decision. Differentiating reactive children who become aggressive from those who remain non-aggressive is clinically vital. Protective factors, such as a supportive environment and positive parenting, may influence the emergence of aggression in some children. However, these children may still face other challenges associated with reactivity, as increased reactivity during childhood has been linked to depression and suicidality during adolescence. Longitudinal monitoring of these subgroups could provide further insights into the development of reactivity, aggression, and other clinical concerns.

Poster 99

The Clinical Utility of Follow-up Allograft Biopsies After Early Acute TCMR In Renal Transplantation

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Introduction: In kidney transplant patients, renal function evaluation by seCr/eGFR is the current gold standard for monitoring response to anti-rejection therapy. How changes in eGFR reflect histological response, and whether follow-up histological evaluation adds prognostic value to clinical assessment by eGFR is unknown.

Methods: We conducted an observational cohort study of patients transplanted between 2013-19 at six transplant centers in Europe and USA and assessed the relationship between ΔeGFR, follow-up allograft histology and 7-yr graft survival in patients with TCMR.

Results: Of the 9000 Bxs from 6000 patients, 866 (14%) had TCMR (42% Banff 1A, 17% 1B, 41% ≥2/mixed TCMR/ABMR) in the 1st post-transplant year. Despite therapy, only 39% of patients exhibited clinical improvement (eGFR improvement to ≤20% of nadir). Similarly, 44% of patients had complete histological TCMR-resolution, whereas 35% had persistent TCMR. TCMR was persistent in 34% of the mild-1A patients, suggesting that histological persistence was not related to rejection severity on index Bx. 26% of patients with clinical improvement had persistent TCMR while 54% of patients without clinical improvement demonstrated either complete or partial histological resolution, suggesting a clinical-histological mismatch. Irrespective of index-histological grade and independent of potential confounders, early histological persistence was associated with significantly worse 7-yr-late AR-free survival and 7-yr-death-censored graft survival. Lack of clinical resolution was associated with significantly worse late AR-free survival or graft survival only when there was early histological persistence of TCMR.

Conclusions: Histological evaluation by follow-up allograft biopsies provides valuable prognostic information after TCMR diagnosis and should be considered in routine clinical practice.

Poster 11

Des-Ciclesonide: A Novel Potential Treatment for Bronchopulmonary Dysplasia with Anti-Inflammatory Effects Against Bleomycin-Induced Lung Injury in Neonatal Rats

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Des-ciclesonide (DES), the metabolite of ciclesonide (CIC), is a potent glucocorticoid receptor (GR) agonist with tissue-specific metabolism and a higher safety profile, compared to dexamethasone (DEX), making it a promising pharmacotherapy for bronchopulmonary dysplasia (BPD).

Sprague-Dawley P0 pups were given five daily s.c. injections of vehicle, 0.5 mg/kg DEX, or 1.25 mg/kg DES. Systemic effects were assessed by measuring weight gain, endpoint brain weight, blood glucose and serum levels of insulin-like growth factor-1 (IGF-1).

DES-treated animals did not exhibit the growth suppression and reduction of IGF-1 levels observed in the DEX animals nor the extent of the decrease in brain weight. While severe hyperglycemia was evident in DEX pups at 4 and 24 hours, DES produced only a modest and transient elevation. RNAseq of lung and liver tissue revealed a greater number and higher level of gene induction in DEX compared to the DES group with unique pathways defined by the transcriptome in each tissue. Nevertheless, DES is as effective as DEX in reducing the expression of various proinflammatory cytokine mRNAs at baseline (e.g. TNF-a) and after lung-injury induced by an 11-day systemic treatment with bleomycin.

DES may be anti-inflammatory without producing the detrimental growth and hyperglycemic effects of DEX in neonatal rats. The potential enhanced safety profile of the prodrug CIC to treat BPD may be influenced by its tissue-selective conversion to a novel selective GR modulator, whose unique transcriptional responses may limit adverse systemic effects typically triggered by therapeutic sGCs in neonates.

Poster 54

Genome-wide investigation reveals suggestive interaction of *TWIST2* with maternal smoking on orofacial cleft risk

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Isolated cleft lip with/without cleft palate (CL/P) is a prevalent congenital birth defect with complex etiology involving both genetic and environmental factors. Despite high heritability, the genetics underlying CL/P remain poorly understood. Here, we explore genome-wide interactions between genetic variants and maternal smoking, an important risk factor for CL/P, within ±3 months of conception.

Our cohort includes 547 Filipinos with isolated CL/P and 264 unrelated controls without family history of clefting. We conducted gene-environment interaction (GEI) analysis on 4.7M variants, combining genotyped (Illumina GDA-8v1-0 array) with imputed variants (using TOPMed reference panel via minimac4) with imputation $R^2 > 0.8$. Using logistic regression, we tested for multiplicative GEI (GxE) on variants with minor allele frequency ≥ 0.10 adjusting for sex and principal components via *GxEScanR*. Additionally, we examined disease-gene (DG) and environment-gene (EG) associations. We evaluated GEI two-ways: (1) via 3 degree of freedom (3-df) joint test combining GxE, DG and EG, and (2) via 2-step approach: In the first step, we screened for DG and/or EG associations with a $p<0.05$ using a joint 2-df test. In the second stage, we tested the GEI effect alone.

We found a suggestive interaction affecting CL/P risk between the intergenic variants near *TWIST2* and maternal smoking. The leading signal rs5839711 ($p_{3df} = 1.55 \times 10^{-5}$, and $p_{G\times E} = 9.34 \times 10^{-5}$) locates upstream of *TWIST2*. *TWIST2* serves as a crucial transcriptional regulator governing the mesenchymal cell fate and epithelial-mesenchymal transition critical for normal embryonic morphogenesis and cancer progression. Notably, *TWIST2* is highly expressed in craniofacial mesenchyme during embryogenesis and mutations in *TWIST2* disrupting these processes lead to clinical and facial features seen in ablepharon macrostomia syndrome and Barber-Say syndrome.

In summary, we found a suggestive interaction near *TWIST2* with maternal smoking associated with risk of CL/P in Filipinos. Further validation is needed to confirm these findings.

Poster 100

Histopathologic Features of Juvenile Onset Localized Scleroderma

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Background

Localized scleroderma is a rare autoimmune skin disorder primarily seen in children. The histopathology of juvenile onset localized scleroderma (jLS) is described poorly due to the rarity of the disease. In this study, we aimed to determine the histopathologic features of jLS.

Methods

We conducted a cross-sectional analysis among 94 skin biopsies from the patients registered in the prospective National Registry for Childhood Onset Scleroderma (NRCOS) cohort. Summary statistics were used for demographic and histologic categorical features. Chi-square analyses were applied to categorical variables.

Results

Of the patient cohort, 71.2% were female (n=67) and the average age of onset was 8.5 years old. The degree of inflammatory was mild (43%, n=41/68), followed by moderate (18%, n=17), and severe (6%, n=6). The degree of sclerosis was scored in only 26% of the biopsies (n=24), with the degree of sclerosis being mild in 9.5% (n=9), moderate in 8.5% (n=8) and severe in 7.4% (n=7). Among the biopsies reporting inflammation, the most common location of inflammatory infiltrates was perivascular (59.5%, n=56). A larger number than expected demonstrated interstitial inflammation (20%, n=19). Correlating the relationship between the validated disease activity measures and degree of inflammation, we found a statistically significant association between the modified Localized Skin Severity Index (mLoSSI) and the degree of inflammation. [X^2 (1, N = 67) = 6.86, p = .0087]

Conclusion

Histopathology reports provide valuable information in localized scleroderma. Especially the degree of inflammation and sclerosis, which augment clinicians' judgment of disease activity and may influence treatment decisions.

Poster 101

MoCA detects pre-clinical AD in the sub-group positive for amyloid and tau pathology

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Cognitively unimpaired (CU) individuals are a heterogeneous group in the Alzheimer's disease (AD) continuum. We hypothesize that sub-categorizing the CU group based on the presence of amyloid (A) and tau (T) pathologies, as measured by PET, can reveal subtle cognitive deficits, using a common clinical tool, Montreal Cognitive Assessment (MoCA), which is traditionally deemed less sensitive for the general CU population. We included 88 CU [defined as CDR global score of 0] older adults with available head-to-head MK6240 tau-PET and Flortaucipir tracers from the ongoing HEAD multi-site observational study. They also underwent Amyloid PET imaging using PiB or NAV4694. MoCA testing was done around the imaging time. Amyloid and Tau PET positivity was defined using visual reads. Based on tracer uptake status, we categorized the individuals into A-T-, A-T+, A+T- and A-T- groups. Unpaired t-test and one-way ANOVA were used to test for significant differences between groups. 2-tailed p-values were significant between the A-T- group and the A+T+ group for MoCA total score [p: 0.0025 and p: 0.0025]. When we stratified MoCA by different cognitive domains, we found that the results were driven by MoCA (memory) Delayed Recall score [p: 0.0007 and p: 0.0162]. A-T+ vs A+T+ was also significant [p: 0.0099] for MoCA Delayed Recall. Both tau tracers (MK6240 and Flortaucipir) showed similar performance for determining T+ and in identifying cognitive decline. MoCA, a simple and commonly administered in-office neuropsychological test, can detect subtle cognitive dysfunction in CU older adults who are Aβ and tau positive (A+/T+). These CU A+/T+ individuals are likely on the path to developing AD dementia.

Poster 55

RIMOC1, a novel regulator of mitochondrial function, and its role in pancreatic beta-cell survival, mitophagy, and diabetogenesis

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Background: Literature supports preserving beta-cell function as an approach to slowing diabetes progression. Mitochondria emerge as an interesting area of focus, driving glucose-stimulated insulin secretion (GSIS) from beta-cells and leading to increased intracellular ROS generation when damaged. Damaged mitochondria are recycled via lysosomes through mitophagy, and defects in mitophagy have been linked to beta-cell death. Our previous work had isolated RIMOC1 (RAB7A-Interacting MON1-CCZ1 Complex Subunit 1) as a protein highly expressed in pancreatic beta-cells, though its role remains unknown. The overarching hypothesis is that RIMOC1 is protective against the development of diabetes by preserving beta-cell mitophagy and mitochondrial function, and that impaired RIMOC1 function would lead to beta-cell failure and diabetes.

Methods: Beta-cell specific knockouts of RIMOC1 were generated in a stable INS2 cell line and INS1-Cre RIMOC1-KO mouse model. Mitochondrial membrane potentials were measured using TMRE/ JC1 stains, and mitophagy was quantified using mtKeima. Ongoing studies include GTT (glucose tolerance testing) and GSIS experiments.

Results: Published GEO datasets show RIMOC1 expression is positively correlated with beta-cell maturity and negatively correlated with glucose load and HgbA1c. We've demonstrated RIMOC1 has selective expression in mouse pancreatic islets. RIMOC1 knockout leads to decreased insulin mRNA expression, decreased insulin content, and increased cell-cycle inhibition in beta-cells. RIMOC1-KO exhibited worsened hyperglycemia with oral refeeding after fasting in mice. RIMOC1-KO displayed significantly diminished mitochondrial membrane potential and mitophagy.

Conclusions: RIMOC1 is highly expressed in beta-cells and has a significant role in maintaining euglycemia through mitochondrial functioning. This research highlights RIMOC1 as a promising drug target for diabetes.

Poster 56

The Lack of Phosphorylation in Amelogenin Leads to Acidification of forming Dental Enamel

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Introduction: Enamel is the most highly mineralized tissue of the human body, made of densely-packed and well-arranged hydroxyapatite (HA) crystals. During its formation, it is composed predominantly of an organic matrix. The enamel matrix proteins (EMPs) are crucial to reaching enamel's thickness and unique crystalline structure, although their precise roles have not yet been fully elucidated. The most abundant of these proteins is amelogenin (AMELX). It has one phosphorylation located on Serine 16, that is essential in controlling enamel formation. Earlier *in vitro* studies showed that phosphorylated AMELX stabilizes amorphous calcium phosphate and prevents HA crystal formation [1,2]. *In vivo*, in a mouse model $AMELX^{Ser16Ala}$ knock-in (KI) which lacks AMELX phosphorylation, apatite crystals form faster and the enamel structure of KI mice is severely affected [3].

Objective: To test the hypothesis that accelerated enamel mineralization in $AMELX^{Ser16Ala}$ KI mice induces local acidification that also affects forming enamel mineral composition.

Methods: Mandibular incisors from 8-week-old wild-type (WT) and KI mice were isolated. They were freeze-dried and immersed in bromocresol purple (BCP) pH indicator to visualize pH differences in forming enamel of KI and WT. To assess the structural differences in enamel mineral composition, we conducted FTIR microspectroscopy in reflectance mode.

Results: BCP staining showed the secretory-stage enamel was more acidic in KI vs. WT incisors. A distinct pattern of alternating low and high pH bands typical of the maturation-stage was absent in KI enamel. Consistent with a higher initial mineral density in KI enamel (unpublished), the mineral to protein ratio was greater in KI secretory stage enamel. The ratio of acidic phosphate to phosphate was also higher in KI enamel, consistent with mineralization in an acidic environment. Together, our observations demonstrate that the lack of AMELX phosphorylation leads to acidification of secretory enamel and affects its mineral and organic composition.

Poster 57

The genetic basis of commensal bacteria-mediated protection against plant pathogens

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Host-associated microbiomes confer crucial benefits to their macroorganisms including improved nutrient acquisition, growth promotion, immune system development, and protection against pathogens. Microbiome manipulation is promising for preventing and treating pathogen infection, although the mechanisms underlying microbiome-mediated pathogen protection remain poorly understood. To examine the genetic basis of pathogen protection, we make use of a genetically tractable host-pathogen-microbiome model consisting of the model plant *Arabidopsis thaliana* (Arabidopsis) and the commensal *Pseudomonas fluorescens* WCS365 that protects Arabidopsis from the closely related pathogenic *Pseudomonas fluorescens* N2C3. We performed a high-throughput 96-well plate *in planta* screen of a WCS365 transposon mutant library that display increased or decreased surface attachment and identified mutants that no longer protect against N2C3. A subset of candidate genes have not been implicated in host-association including *wbpA* and *wpbD*, genes responsible for lipopolysaccharide O-antigen biosynthesis, and *rpfC*/RS23125, a two-component signaling system. Using genetics, RNAseq, and *in vitro* and *in planta* assays, we will uncover whether these genes confer protection via promoting WCS365 plant colonization, direct antagonism against N2C3, and/or modulation of plant immunity. This research will reveal the genetic underpinnings of pathogen protection mediated by commensal bacteria to further our understanding of how microbiomes function. As microbiome manipulation could prevent and treat disease, elucidating these mechanisms is essential for food security and human health.

Poster 58

Designing reporters for multiplexed, real-time monitoring of membrane protein targeting fidelity

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Maintaining the integrity of eukaryotic organelles relies on targeting membrane proteins to the correct organelle. A specific category called Tail-anchored (TA) proteins is directed to diverse organelles such as the endoplasmic reticulum (ER), mitochondria, and peroxisomes. TA proteins are characterized by a cytosolic N-terminal domain followed by a single transmembrane domain (TMD) at its C-terminus. Due to the presence of specific targeting sequences at the C-terminus, TA proteins are subjected to post-translational targeting. Despite the sophistication of the targeting pathways, errors regularly occur, even under non-stressed conditions. Such failure in maintaining proper protein localization is implicated in a range of diseases, including neurodegeneration, cancer, aging, and cystic fibrosis. Although numerous studies have suggested that TA-protein targeting is prone to errors, the precise magnitude of this intrinsic error rate in mistargeting is elusive because there is a lack of reporters capable of real-time monitoring of protein targeted to different organelles. To circumvent these challenges, we have developed a reporter using the nano-lanterns in combination with fragment complementation of Nano-Luciferase (split-BRET). With the developed ER nano-lantern reporter system we have effectively distinguished between ER and mitochondrial substrate targeting. We are now in the process of expanding our reporters to organelles like mitochondria and peroxisomes.

Poster 102

ECG-Based Risk Stratification of Pulmonary Embolism

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Background: Pulmonary embolism (PE) carries significant risk of morbidity and mortality, but current clinical algorithms for risk stratification of PE require several imaging and laboratory studies that can delay identification of the highest risk individuals. We sought to examine whether machine learning models using the 12-lead electrocardiogram could identify patients with elevated risk.

Methods: This was a single-center retrospective study of consecutive patients who were diagnosed with PE within 2 days of admission. Patients who had a 12-lead EKG performed within 1 day of PE diagnosis were included. PEs were categorized as massive or sub-massive (together, elevated-risk) or low-risk at the time of clinical evaluation, based on the PERT Consortium guidelines. ECG features describing the morphology, beat-to-beat variability, spatial projection, and power spectral density of the ECG were computed. Machine learning models were trained on a selected feature set, and performance was evaluated on a hold-out test set.

Results/Data: 1,248 patients were included (mean age 59 years, 49% women, 55% elevated risk). The 419 ECG features were narrowed to the 135 highest importance features. A random forest model trained on these features identified elevated-risk PE with an AUROC of 0.84 on the hold-out test set, outperforming existing ECG-based risk scores. At the selected operating point, the model achieved 74% accuracy, 83% sensitivity and 63% specificity. The features selected by the model included known findings of PE, as well as novel features of ST-segment slope and T-wave morphology dispersion. In addition, examination of clinical outcomes suggest that model risk predictions better predict 90-day mortality than the 'true' clinical risk classification.

Conclusions: This work demonstrates that a machine learning model using ECG morphology-derived features can identify patients with elevated clinical risk from PE. ECG-based screening of patients diagnosed with PE could provide rapid identification of the highest risk patients before further clinical workup is completed.

Poster 119

Endogenous modifications in motor cortical activity allows online error detection in human Brain-Computer Interfaces

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The motor cortex is known to encode future motor commands. Decoding motor intents from population activity in the M1 area is at the basis of brain-computer interfaces (BCIs). However, how overall activity in M1, and more specifically the mapping between population activity and motor commands, are perturbed by feedback signals during a motor task remains unknown. To study this, we analyzed data from 3 human participants with tetraplegia controlling a BCI to perform a continuous 2D cursor control task and receiving visual feedback of the cursor movement. Epochs of correct and erroneous control feedback are defined based on whether the controlled cursor appears to move on or off-target. First, we highlight significant differences in the population activity of M1 between periods of correct and erroneous control: we show that the subspaces of neural activity spanned during these periods are significantly misaligned, and that neural activity during erroneous epochs is characterized by a dimensionality collapse. But whether these population activity changes are the cause of control errors, or the consequence of receiving feedback of the task output, is still unclear. We show that dimensionality collapse tends to precede the onset of control error, supporting the hypothesis that these errors are caused by endogenous changes in the population activity of M1 rather than by a sensitivity to task feedback. Finally, using these activity changes as a neural signature of ongoing error, we trained a decoder to detect them: this allows to perform online error detection during BCI control to stop ongoing errors without any specific action from the participant. Overall, our results highlight the role of the motor cortex as an optimal controller, leveraging separately upstream target and feedback loop signals to compute downstream control commands.

Poster 12

Sexually dimorphic Atf4 expression and activity influence adipose tissue physiology in *Drosophila melanogaster*

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Metabolic differences between males and females have been well documented across many species. However, the molecular basis of these differences and how they impact tolerance to nutrient deprivation is still under investigation. In this work, we use the insect *Drosophila melanogaster* to demonstrate that sex-specific differences in fat tissue metabolism are driven, in part, by dimorphic expression of the evolutionarily conserved Integrated Stress Response (ISR) transcription factor, Atf4. We found that female fat tissues have higher Atf4 activity than their male counter parts under homeostatic conditions. This dimorphism was partly due to a female bias in *Atf4* transcript abundance in fat tissues and driven by canonical sex determinants: masculinization of female fat tissues via depletion of the sex determinants *transformer*, *doublesex*, or *spenito* alters the relative abundance of *Atf4* splice isoforms. These differences persist under stress conditions, where female fat tissues show substantially higher Atf4 induction than males in a genetic model of nutrient deprivation, indicating that higher Atf4 activity confers higher tolerance to nutrient deprivation in females. Finally, we demonstrate that dimorphic ISR activity in fat tissues confers a metabolic advantage that increases stress tolerance into adulthood. Genetic induction of nutrient deprivation caused developmental lethality disproportionately in males. We are currently testing the dependence of this sex-specific viability defect on ISR pathway activation. Together, our data describe a previously unknown facet of ISR signaling wherein sexual identity of adipose tissue confers differential stress tolerance in males and females. Future work will investigate functional differences between Atf4 isoforms and how they inform sex differences in fat tissues. Since Atf4 promotes cellular function and survival under homeostasis and stress, our studies will shed light on how sexual identity influences metabolism in both homeostasis and disease.

Poster 59

Elucidating the pathobiology of tracheostomy-associated tracheobronchitis (TATB)

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Every year over 100,000 Americans undergo tracheostomy due to critical illness, resulting in acute care costs exceeding $11 billion. Approximately 30% of tracheostomy patients experience respiratory illness characterized by increased sputum secretions, but without radiographic infiltrates. These symptoms are attributed to "tracheostomy-associated tracheobronchitis (TATB)". Studies suggest that TATB prolongs recovery and delays ventilator-weaning, yet its underlying causes are poorly understood.

To study the pathology of TATB, we have assembled a prospective cohort of patients requiring prolonged ventilator support in long-term acute care hospitals (LTACH). The LTACH population is severely understudied for respiratory infections and is prone to developing antibiotic resistance. Combining microbial genomics with host inflammatory profiling, we will test the hypothesis that TATB is an infectious event that triggers an inflammatory response, thus delaying decannulation and results in worse outcomes.

Traditional pathogen identification via 16S rRNA amplicon sequencing is limited to genus-level taxonomic profiles. Thus, we used an innovative approach, leveraging Oxford-Nanopore long-read sequencing of the full-length 16S rRNA gene to identify microbial species in the tracheal secretions.

Our pilot study demonstrates the reliability of full-length 16S long-read sequencing in providing microbial species-level information. Although most tracheal secretions harbor diverse oral microflora, some samples were dominated by the pathogen *Pseudomonas aeruginosa*. Thus, our preliminary results reveal that even though all participants tested had high secretion burden, not all of them were colonized by pathogens.

Expanding our full-length 16S rRNA approach to analyze all LTACH samples, we will correlate 16S community analysis with cytokine marker profiling to assess local inflammation and predict active infections. These analyses will be clustered with the participant metadata- timing of antibiotic administration and time to decannulation, to study the effect of TATB on overall participant recovery.

Poster 60

A non-canonical mechanism of antiviral activity of Oligoadenylate Synthetase 1

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The translational arrest of protein synthesis is often a cellular response to the virus infection. However, some antiviral proteins continue to be translated through unknown mechanisms during this translational shutdown. Here, we report a mechanism by which antiviral effectors are upregulated during this translational shutdown. We found that the interferon (IFN) stimulated gene, Oligoadenylate Synthetase 1 (OAS1), binds AU-rich elements (ARE) of specific mRNA, including IFNβ, prolonging the half-life and continued expression. This increased IFN expression protects from WNV infection *in vitro* and *in vivo* via downstream IFNAR signaling. This mechanism is common between human OAS1 and mouse Oas1b, independent of OAS enzyme activity and RNase L. However, human OAS1 inhibits SARS-CoV-2 replication through its canonical enzyme activity via RNase L, thus establishing two different mechanisms of OAS1 antiviral activity. These results establish OAS1 as an ARE-binding protein with a broader non-canonical function that protects IFN expression from translational shutdown.

Poster 13

Surgery-induced Neutrophil Extracellular Traps reprogram cancer cell metabolism leading to tumor progression.

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Introduction: Although surgery is a crucial intervention to cure malignancies, its accompanying immune response, including a systemic release of Neutrophil Extracellular Traps (NETs), can enhance tumor progression. We aimed to explore the direct effects of surgical NETs in regulating cancer metabolism leading to tumor growth and metastasis.

Methods: C57BL/6 wild-type or PAD4-KO mice (n=10) underwent subcutaneous or intravenous cancer injections (LLC-1 and MC-38), followed by midline laparotomies, with or without perioperative NETs inhibition (DNAse or GSK484).

Results: Mice subjected to laparotomy had significantly increased lung and subcutaneous tumor burden, while perioperative treatment with DNAse or GSK484, or utilizing PAD4-KO mice reversed the surgically-induced tumor growth. Pre-treating cancer cells with NETs *in vitro* prior to inoculation also increased tumor burden. Transcriptomic analysis of MC-38 cancer cells exposed to surgical stress *in vivo*, or treated with NETs *in vitro* showed activation of the MYC oncogenic pathway by NETs. This was associated with upregulation of fatty acid oxidation (FAO), shown with METAFlux metabolic profiling and Acetyl-CoA analysis. Furthermore, blocking FAO with etomoxir (CPT1α inhibitor) reversed the cancer proliferation and O2 consumption induced by NETs, as well as the tumor growth and metastasis *in vivo*. FAO activated by NETs was also crucial for survival of circulating cancer cells in a model of anoikis stress. These findings were corroborated in human tumors analyzed from the TCGA database.

Conclusion: Surgical insult promotes tumor progression through cancer cell metabolic reprogramming by systemically released NETs. Inhibiting the underlying molecular mechanism may therapeutically prevent the protumorigenic effects of surgical stress.

Poster 61

Insights of local ancestry from an orofacial cleft genome-wide association study in a Filipino cohort

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Orofacial clefts (OFCs) are the most common birth defects among craniofacial dysmorphologies. OFCs are predominantly non-syndromic and manifest with no other symptoms. Occurrences of these birth defects are highly variable between population groups with the highest incidence observed within individuals of Asian descent. This highlights the importance of expanding population-based studies by including multi-ancestry approaches into current OFC research to address craniofacial health disparities and risk factors. Here, we hypothesize that adjusting for measures of genome-wide (global) and locus-specific (local) ancestry will improve identification of OFC risk loci. We implemented several genetic ancestry approaches to measure and visualize the genetic variance within a cohort of Filipino participants recruited throughout the Philippines and demonstrate the close genetic relationship between this cohort and East Asian individuals. To systematically compare the impact of full ancestry adjustment in genetic association studies, including both global and local ancestry as covariates, we first performed a genome-wide association test for non-syndromic OFCs adjusting only for sex and global ancestry. Then, we implemented a local ancestry inference pipeline, Tractor, to perform a two-way admixed model while also considering sex and global ancestry. The lead loci from the first genome-wide association test were replicated, but we observed an increased number of significant associations that could represent ancestry-specific loci associated with non-syndromic OFCs. Although this method shows promise in future OFC studies, we observed in our cohort less balanced admixture ratios than in previous Tractor simulations leading to the conclusion that the ancestry-specific effect of one ancestry heavily outweighed the other. We suggest this approach in future OFC population studies involving individuals that possess more balanced admixture ratios of two or more genetic ancestries, which could reveal additional ancestry-specific OFC risk loci.

Poster 36

Effect of Ketamine on Individual Symptoms of Depression in a Randomized Controlled Trial of Ketamine for Treatment-Resistant Depression

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Intravenous ketamine has emerged as an effective rapid acting treatment for treatment resistant depression (TRD). However, the precise mechanism of action of ketamine is not understood yet. Moreover, not all patients respond to ketamine, and it remains unclear who is likely to respond to ketamine. The difficulty in understanding the mechanism of action of ketamine and its clinical effects might be related to the heterogeneity of TRD symptom profiles. An understanding of ketamine's differential effects on depressive symptoms could provide insight into these issues. In this study, 152 individuals with TRD were randomized to receive ketamine or saline infusion. Clinician-ratings of depressive symptoms were assessed at multiple time points from pre-infusion to 30 days post-infusion using Montgomery–Åsberg Depression Rating Scale (MADRS), and their differences on the symptom level were calculated. Also, in order to study how ketamine affects the relationship between depressive symptoms (i.e., the mutual effect of a pair of depressive symptoms on each other), we estimated the depressive symptom networks with Gaussian Graphical Models (GGM) at pre-infusion and 24-hrs-post-infusion. Compared to saline infusion, the greatest effects of ketamine at 24-hr post-infusion were on symptoms of apparent sadness, reported sadness, sleep problems, and numbness (ds>0.2, p<0.05). For certain symptoms such as lassitude, the greatest improvement in symptoms was found on day 5 (ds>0.5, p<0.05), and suicidality showed maximal improvement later in treatment on day 21 and day 30 ($d_{day\ 21}$=0.44, p<0.05). The symptom networks did not reveal an overall network structure change pre- to post-infusion; however, the global strength of the network post infusion increased significantly, suggesting a denser symptom network 24-hrs post infusion. Moreover, the association between apparent sadness and reported sadness increased. Overall, ketamine appeared to improve different symptoms at different time scales and it might affect some symptoms directly while others indirectly through other symptoms.

Poster 62

A model of shell structure and pattern in mollusks

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A continuous space-discrete time neural model is proposed to generate diverse shell structures and pigmentation patterns on aquatic mollusks. We employed a system of nervous excitation and inhibition of secretory activity to reproduce some of the existing shell patterns observed on aquatic mollusks. The response of the neuron cells is non-linear. The analysis of local stability and bifurcation predicts how the change in shell patterns occurs. The general patterns in shell are the results of three types of bifurcation: Turing bifurcation, Hopf bifurcation and Turing-Hopf bifurcation. More complex Turing patterns or oscillating patterns can be generated by considering different non-linear function.

Poster 63

Post-natal Prx1 Expressing Cells Contribute to Periodontal Regeneration

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Objectives: Post-natal skeletal stem cells expressing Paired related homeobox 1 (pnPrx1 cells) are known to play an important role in bone regeneration. We have previously reported that pnPrx1 cells are required for the regeneration of the mouse periodontal ligament (PDL). However, whether pnPrx1 cells directly contribute to the regeneration of the PDL remains unclear. In the present study, we aimed at identifying the contribution of pnPrx1 cells to the periodontal regeneration of the mouse molars.

Methods: Periodontal disease was induced in mice by placing a 5-0 silk ligature around the left maxillary second molars and keeping it in place for 7 days. Six male mice (Prx1-CreEr-GFP+/TdTomato+), 6–8 weeks old, were randomly divided into two groups. One group was euthanized 7 days after ligature removal, while the other group was euthanized 14 days after ligature removal. The right maxillary molars were used as healthy controls. To perform lineage tracing analysis, all mice were injected with tamoxifen 2 days pre-ligation, and every 48 hrs thereafter, until the ligature was removed. 7- and 14-days after ligature removal, maxilla were harvested for histological evaluation and for fluorescence microscopy detection of TdTomato expressing red-fluorescent cells (TdTomato+).

Results: 7-days after ligature removal, the PDL was disrupted and limited periodontal regeneration was observed. Few TdTomato+ cells were observed in the newly formed PDL and bone. 14-days after ligature removal, the PDL is regenerated and a significantly higher number of TdTomato+ cells were observed in PDL, including the furcation PDL, and the surrounding bone. No TdTomato+ cells are observed in control right side at 7- or 14-days after ligature removal.

Conclusion: PnPrx1 cells directly contribute to the regeneration of the PDL of the mouse molars. Additional studies are being performed to evaluate whether these cells can be harnessed to foster periodontal regeneration.

Poster 64

Pivotal Role of MET in Promoting Liver Regeneration Following Acetaminophen Hepatotoxicity Identified Using Liver-Specific Knock-Out and Pharmacological Inhibition Strategies in Mice

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Introduction: MET, the receptor for hepatocyte growth factor (HGF), drives liver cell proliferation post partial hepatectomy (PH). Its involvement in acetaminophen-induced liver injury (AILI) and regeneration remains unclear, despite (Acetaminophen) APAP overdose being a primary cause of acute liver failure. MET's role in AILI differs from its function in PH due to distinct regenerative responses shaped by massive injury and inflammation. Notably, our prior study showed dose-dependent MET activation in mice post-APAP overdose, particularly pronounced after severe toxicity.

Methods: We explored MET's role in AILI and regeneration using two methods: systemic administration of the c-MET inhibitor Capmatinib (15 mg/kg) and liver-specific MET deletion via an albumin-CRE and AAV8-TBG-CRE system. All mice received 300 mg/kg of APAP to assess liver injury, regeneration markers, and signaling cascade at various time points.

Results: Systemic MET inhibitor administration did not affect initial liver injury but suppressed hepatocyte proliferation post-APAP overdose in mice. Liver-specific MET KO mice also showed decreased hepatocyte proliferation compared to WT mice. MET KO mice experienced severe impairment in liver regeneration, leading to uncontrolled injury progression and significant mortality, while WT mice recovered fully. Although the mechanisms initiating AILI remained unchanged after MET inhibition or deletion, ERK signaling was consistently inhibited in both cases. This inhibition correlated with suppressed cyclin D1 induction and failed activation of cell cycle machinery, impairing liver regeneration. RNA sequencing and Ingenuity Pathway Analysis indicated failed activation of key regulators of hepatocyte proliferation upon MET signaling disruption.

Conclusion: Pharmacological MET inhibition or liver-specific MET deletion hindered hepatocyte proliferation and impeded liver regeneration post-APAP overdose. These findings underscore the critical role of MET in the recovery from APAP-induced acute liver failure.

Poster 65

Intravenous BCG Elicits a More Robust Immune Response in Macaques Infected with Simian Immunodeficiency Virus than Intradermal BCG.

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is one of the leading causes of death due to an infectious agent. Coinfection with Human Immunodeficiency Virus (HIV) exacerbates Mtb infection outcomes in people living with HIV (PLWH). A TB vaccine that is safe and effective in PLWH would be enormously beneficial. Bacillus Calmette-Guérin (BCG), the only approved TB vaccine, is administered by intradermal (ID) injection and is effective in infants, but its efficacy wanes in adolescents and adults. We recently showed that BCG given by the intravenous (IV) route to macaques with preexisting Simian Immunodeficiency Virus (SIV) conferred robust protection from TB. Here, we compared the immunological responses evoked by IV or ID administration of BCG in cynomolgus macaques infected with SIV. We evaluated the effect of vaccination on T cell responses in the airways, blood, and tissues (spleen, thoracic lymph nodes [ThLN], and lung), including expression of transcription factors, cytokines, and cytotoxic molecules. We delineated vasculature (ivCD45+) from tissue-resident memory T cells (T_{RM}) (ivCD45-) by injecting anti-CD45 antibody via IV prior to necropsy. We also evaluated the antibody response in both blood and airways. Our data show that BCG IV triggers a strong and long-lasting immune response. BCG IV elicited a high number of T cells in the lung, including T_{RM}, polyfunctional CD4+ and CD8αβ+ T cells, and CD8αβ+ T cells and NK cells that produce cytotoxic molecules. We also found higher levels of mycobacterial-specific IgG and IgM in airways following BCG IV. Importantly, these responses were impaired in animals with high levels of SIV RNA, highlighting the importance of viral control for optimal responses to BCG vaccination. In macaques with good SIV control, BCG IV was safe and more immunogenic than BCG ID.

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Poster 66

Hypermetabolic expansion conditions imprint lasting dysfunction on adoptive cell therapies.

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Generating required cell numbers is a limiting factor in adoptive cell therapies like chimeric antigen receptor (CAR) T cell therapy, leading to the development of bioreactors designed for high scale proliferation. Culture conditions used for T cell expansion are *extremely* hypermetabolic, often *2 to 10 times richer* in fuel sources like glucose compared to physiological levels. This may result in dysfunctional cells unable to persist and function in fuel deficient *in vivo* environments. Thus, we hypothesize that commonly used hypermetabolic expansion conditions contain high amounts of nutrients like glucose and may push T cells towards terminal differentiation, resulting in poor anti-tumor response and impaired memory formation *in vivo*. To compare the efficacy of commonly used T cell expansion conditions, Peripheral blood mononuclear (PBMCs) cells were used to generate anti-CD19 CAR-T cells in RPMI with increasing amounts of glucose (5mM, 1mM, 55mM) in gas-permeable Rapid expansion $(G\text{-}Rex)_R$ bioreactor or traditional flasks and analyzed for metabolic/functional parameters. T cells expanded in hypermetabolic conditions showed decreased mitochondrial capacity indicative of metabolic insufficiency and poor *in vivo* persistence. Additionally, CAR T cells expanded in physiological 5mM glucose activated better and were more functional *in vitro* and *in vivo* compared to cells expanded in hyperglycemic media in a NALM6 leukemia model. Our data suggests that commonly employed hypermetabolic culture conditions may imprint an unappreciated form of dysfunction. This suggests that modifying or engineering cell culture systems to more adequately mimic physiologic metabolic conditions may better prepare T cells to eradicate cancer in patients.

Poster 103

Title: Quality of life in hospitalized patients with Alcohol-related Acute or Recurrent Acute Pancreatitis

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Background and Aims:
Alcohol abuse is among the most common causes of acute (AP) and recurrent acute pancreatitis (RAP). Few data exist on the impact of hospitalization on quality of life (QOL) in patients with alcohol-related AP or RAP, improvement in QOL after resolution of an AP episode and factors that associate with poor QOL. Our aim was to define the QOL in patients with alcohol-related AP and RAP and factors associated with QOL in these patients.

Methods:
We analyzed data during hospitalization in 138 subjects with alcohol-related AP or RAP prospectively enrolled in an observational US multicenter study (ACCESS-AP). Diagnosis and severity of AP were defined by the Revised Atlanta Classification and a score of >=3 on AUDIT-C scale was required for eligibility. Subjects completed a self-administered instrument for QOL assessment (PROMIS 29); based on their responses T-scores were calculated for Physical and Mental QOL using on a scoring algorithm. A trained coordinator interviewed subjects to record detailed information on symptoms, alcohol, tobacco and substance use and psychological comorbidities. Clinical data were collected from electronic health records. Multivariable linear regression analyses using backward elimination evaluated independent associations for QOL.

Results:
Mean age at enrollment was 41.5 years, 69% were male, 51% were white, 80% were non-hispanic, 67% were ever smokers (51% current), 68% ever used marijuana (33% current), 53% had history of anxiety or depression and 56% had prior AP. Moderate or severe AP was noted in 26% and 16% needed ICU care. Mean T-scores for Physical (29.21±7.9) and Mental (38.4±3.6) QOL were more than 2 and 2 standard deviations below population mean (50±10; clinically significant difference: >=3 points). On multivariable analyses, clinically significant differences in physical QOL were noted for a history of diabetes, depression and disease severity, and for none of the patient and disease-related factors for mental QOL.

Conclusion:
Physical and Mental QOL is profoundly affected in patients hospitalized for alcohol-related AP or RAP. Ongoing analyses of longitudinal data for ACCESS-AP study will help evaluate whether QOL improves after resolution of the AP episode and factors are associated with persistently poor QOL after AP.

Poster 14

Combined targeting of synthetic lethal partners in RB1-deficient cells

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RB1 is a tumor suppressor gene that is frequently mutated in various tumors, including retinoblastomas, small cell lung cancers, triple-negative breast cancers, prostate cancers, and osteosarcomas. *RB1* is one of the most prevalent tumor suppressor genes driving metastasis. One therapeutic strategy for treating cancers with inactivated *RB1* involves synthetic lethality (SL). A pair of genes can be defined as synthetically lethal when perturbation of either gene alone is not lethal but simultaneous perturbation of both becomes lethal. We performed a genetic screen for SL partners of Rb in the *Drosophila* eye and confirmed the validity of identified targets (splicing machinery, RAN, eIF4A3, and others) in human cancer cell lines and patient tumor samples. Furthermore, these SL interactions are preserved in the presence of additional oncogenic alterations (activation of Ras and loss of Pten). It is unlikely that monotherapy will be effective for eradication of *RB1*-mutated tumors, thus a combined targeting of two SL partners from different pathways is proposed for a synergistic effect. We created or obtained five pairs of isogenic cancer cell lines (prostate, lung, breast cancer), where RB1 is knocked out or downregulated. We further identified a library of 125 drugs against either SL partners or associated pathways. We screened drugs independently for selectivity against *RB1*-deficient cells and identified several potential candidates, which will be tested in pairwise combinations. For the best combinations, we will dissect downstream mechanism responsible for increased selectivity. We aim to identify the most effective pair of FDA-approved drugs that selectively kills *RB1*-deficient cancers.

Poster 15

Inflammatory and oxidative stress responses to arduous military training in men and women: associations with stress, sleep disturbance, and fitness test performance

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Background: Arduous military training consists of intense physical activity, sleep disturbance, and stress that leads to high rates of musculoskeletal injury (MSKI) risk and causes performance decrements. Inflammatory and oxidative stress increases have been reported in response to arduous training, but with inconsistencies across markers and with under-representation of women. Our purpose was to characterize inflammation and oxidant responses to military training and to correlate biomarkers with subjective measures of stress and sleep quality as well as military fitness test performance.
Methods: Candidates undergoing the 10-week Marine Corps Officer Candidate School (OCS; 101 men; 62 women) were monitored, with demographic and questionnaire data collected, and blood drawn before and after OCS. Military fitness tests were completed going into, during, and following OCS. Blood was analyzed for six markers of inflammation and three markers of oxidative stress. Pearson correlations between biomarkers, questionnaires and fitness test performance were performed.
Results: Following OCS all measured cytokines (IL-6, CRP, TNF-α, IFN-γ, IL-8, and IL-10) were elevated, with women having greater concentrations of several markers. Sleep disturbance and stress perception were associated with IL-6, IL-10 and CRP concentrations in a matter suggesting that high sleep disturbance and stress perception were associated with high inflammatory load. Additionally, those with the highest levels of inflammation at each time point performed worse on fitness tests than those with low inflammation.
Conclusion: Ten-weeks of military training caused an inflammatory response that resembles chronic low-grade inflammation. This circulating inflammatory environment appeared worse with poor sleep, high stress perception, and poor fitness test performance, with utility observed for CRP, IL-6, and IL-10 as biomarkers of these responses. Since inflammation may contribute to MSKI and performance decrements, strategies to minimize inflammatory spikes during training should be explored.

Poster 16

Advancing Peripheral Nerve Recovery: The Role of Bioengineered Tissue Wraps

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Peripheral nerve injuries (PNIs) significantly disrupt motor and sensory functions, often leaving lasting deficits. Traditional repair techniques have limitations, necessitating the development of innovative treatments to enhance recovery outcomes. This study investigates the effectiveness of novel bioengineered wraps made from human amniotic and umbilical tissues in facilitating the regeneration of sciatic nerves in a Lewis rat model.

Employing a comparative analysis, rats underwent sciatic nerve transection followed by either standard surgical repair (control) or repair augmented with tissue wraps (treatment groups). Recovery metrics included cold nociception, mechanical and pressure sensitivity, motor function (via Sciatic Function Index), and muscle atrophy assessment over a 12-week period.

Results revealed that both amniotic and umbilical wraps significantly accelerated sensory recovery without compromising motor function. Specifically, the amniotic wrap group exhibited sustained pressure sensation, faster normalization in punctate sensitivity, and indications of motor function improvement. Although muscle atrophy was evident in all groups, those treated with amniotic wraps showed lesser atrophy, highlighting the wraps' potential in mitigating muscle loss post-injury.

In conclusion, bioengineered amniotic and umbilical tissue wraps represent a promising avenue for enhancing PNIs recovery, offering expedited sensory recovery and potential motor function improvements. These findings advocate for further research into optimizing such bioengineered solutions for peripheral nerve repair.

Poster 67

SRY Drives the Male-Specific Susceptibility to Acetaminophen-Induced Liver Injury

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Background and Aims: Overdose of acetaminophen (APAP) is the major cause of acute liver failure with a male predisposition. We have previously reported that the sex-determining region on the Y chromosome (SRY), a male-specific gene, can promote the development of hepatocellular carcinoma and liver ischemia/reperfusion (I/R) injury. Here, we aimed to determine whether and how SRY also affects drug-induced liver injury (DILI).

Methods: We constructed hepatocyte-specific SRY-overexpressing transgenic (Sry-TG) and hepatocyte-specific SRY knockout ($Sry^{\Delta HC}$) mice and subjected them to APAP-induced DILI model. Hepatocytes isolated from Sry-TG mice were isolated and treated with APAP to determine the in vitro effects of SRY. Multiomics analysis was used to explore the molecular mechanisms by which SRY affects DILI.

Results: SRY was found to be markedly upregulated in the livers of both male patients and mice subjected to DILI. Sry-TG mice exhibited significantly augmented APAP-induced liver injury and activated apoptosis and oxidative stress in vivo and in vitro. In contrast, the male $Sry^{\Delta HC}$ mice showed a protective phenotype. Mechanistically, RNA-seq revealed that Gstm2/3 genes expression were downregulated in the hepatocytes of Sry-TG mice. Liquid chromatography-tandem mass spectrometry (LC–MS/MS) identified PARP-1 as the candidate protein that interacts with SRY. SRY enhances the nuclear accumulation of PARP-1 and stabilizes PARP-1 by recruiting ubiquitin specific protease 7 (USP7) to prevent PARP-1 ubiquitination. PARP-1, in return, suppresses Gstm2/3 mRNA expression by PARylating C/EBPβ and inhibiting C/EBPβ-mediated transcriptional activation of Gstm2/3, leading to the suppression of glutathione metabolism.

Conclusions: Our results suggest that a higher level of SRY expression in males may have accounted for the susceptibility of male mice to APAP-induced liver injury. Inhibition of PARP1 may represent a promising therapeutic approach to treat DILI in males.

Poster 17

Spatial landscape of malignant pleural and peritoneal mesothelioma tumour immune microenvironment

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Malignant mesothelioma is an aggressive cancer with around 3000 new cases diagnosed in the U.S. yearly. A deeper understanding of composition of the tumor immune microenvironment (TIME) with spatial distribution of the immune cell is needed to dissect the interactions between tumor and immune cell subtypes that might help to revisit and improve the efficacy of currently used and potential immunotherapy strategies and extend overall survival.

To elucidate the spatial distributions of major immune cell populations in patients with malignant pleural (n=88) and peritoneal (n=25) mesotheliomas (MPM and MPeM, correspondingly), clinical tissue microarrays were characterized with high throughput multiplex immunofluorescence. The assessed TIME cells spatial distributions were analyzed in their association with tumor suppressor genes expression: *LAG3*, *BAP1*, *NF2*, and *MTAP* (*CDKN2A* proxy). We further analyzed the relationships between spatial distribution of immune cell subpopulations with clinical features and patients' survival prognosis.

TIME immune cells distribution within MPM and MPeM was similar. However, there was a higher level of interaction between $CD4^+$ and $CD8^+$ T cells and tumor cells in MPM than MPeM. Within MPM tumors, there was a higher level of interaction between immune cells and $CD8^+$ T cells in "BAP1 high" tumors compared to "BAP1 low" tumors. The identified patterns of cell-to-cell interaction could be potentially implicated in the immune response against the tumor and serve as the factors in the different behaviors of these two types of mesotheliomas.

Based on the histological analyses the Human Atlas of Malignant Mesothelioma at https://mesotheliomaspatialatlas.streamlit.app has been established.

Our study exemplifies the utility of spatial resolution within single-cell analyses in translational research and provides a valuable resource for finding strategy for personalized medicine and targeted therapy of malignant mesothelioma.

Poster 18

The de-sulfinylation enzyme sulfiredoxin-1 attenuates hepatic stellate cell activation and liver fibrosis by modulating the PTPN12-NLRP3 axis

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Liver fibrosis is characterized by the progressive scarring of liver tissue. Oxidative stress is a critical causal factor of hepatic stellate cell (HSC) activation and the subsequent liver fibrogenesis, but the detailed mechanism is not fully understood. Cysteine sulfinic acid (Cys-SO_2H), a modification of reactive cysteine residues, is a unique form of oxidative response that alters the structure and function of proteins. Sulfiredoxin 1 (SRXN1) is responsible for ATP-dependent reduction of the Cys-SO_2H to sulfenic acid (Cys-SOH). In this study, we found that the expression of SRXN1 was increased in activated HSCs and in human and mouse fibrotic livers. HSC-specific ablation of *Srxn1* or pharmacological inhibition of Srxn1 exacerbated HSC activation and sensitized mice to liver fibrosis. Mechanistically, SRXN1 inhibited HSC activation by de-sulfinylating the phosphatase protein tyrosine phosphatase non-receptor type 12 (PTPN12), which enhanced its phosphatase activity, leading to decreased tyrosine phosphorylation and activation of the pro-fibrotic inflammasome protein NLRP3. The anti-fibrotic effect of SRXN1 was abolished when NLRP3 was inhibited. In contrast, overexpression of PTPN12 attenuated NLRP3 activation, and this effect was further amplified by the C164A *S*-sulfinylation resistant mutant of PTPN12. Our findings have uncovered an important role of SRXN1 and protein *S*-sulfinylation in HSC activation and liver fibrosis. The SRXN1-PTPN12-NLRP3 axis represents potential therapeutic targets for liver fibrosis.

Poster 104

Blue Light as an Anti-inflammatory and Analgesic Strategy in Thoracic Trauma: Protocol for a Randomized Controlled Trial in Adults with Multiple Rib Fractures

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Introduction: Previous work of the Rosengart laboratory has shown blue light to modulate the immune response in pre-clinical tissue injury models, decreasing inflammation while facilitating pathogen clearance. Clinical feasibility has also been demonstrated in a pilot trial of appendicitis. The immunomodulatory effects of blue light are likely relevant in trauma, where an exaggerated and often damaging inflammatory response persists well beyond the injury's initial threat to life. Furthermore, blue light downregulates cytokines that are causative in pathological pain (i.e., IL-6), suggesting possible analgesic properties.

Methods and Analysis: We propose a randomized controlled trial in which adults (≥18 years) with multiple (≥3) rib fractures will be allocated 1:1 between blue light intervention (442 nm, 1700 lux, 12h/day for 3 days) and ambient light control arms. To test our hypothesis that blue light decreases pain as our primary clinical outcome, pre- and post-intervention pain scores and opioid requirements will be compared. To explore how blue light modifies the immune response to trauma, pro-inflammatory (IL-6, TNF-α) and anti-inflammatory (IL-10) cytokines will be measured pre- and post-intervention using ELISA of blood samples. Sample-size analysis, performed using data from a similar thoracic trauma population, yields 28 participants needed per group to detect a 20% difference in pain scores with 90% power.

Conclusions: Blue light therapy, if demonstrated to have anti-inflammatory and analgesic benefits, may be a valuable minimal-risk, non-pharmacologic adjunct in the non-operative management of rib fractures and related tissue injury states.

Poster 37

Health behaviors in pregnancy are associated with biomarkers of placental health and function

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Introduction

Circulating placental biomarkers are dysregulated in adverse pregnancy outcomes (APO). While health behaviors are associated with APO, it is not well-characterized whether health behaviors impact circulating placental biomarkers. We examined associations between health behaviors and placental biomarkers in early- to mid-pregnancy.

Hypothesis

Pregnant people engaging in healthier behaviors would demonstrate more favorable concentrations of placental biomarkers.

Methods

Participants with available analytes from the Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-be were included (N=2,078). Physical activity (PA), sleep, smoking, and diet were scored from 0-100 according to the Life's Essential 8 algorithm; a higher score is more favorable. Biomarkers assayed included total endoglin (Eng), pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PlGF), and soluble FMS-like tyrosine 1 (sFlt). The association between health behaviors and each biomarker was examined using multiple linear regression. Bonferroni-corrected significance was $p<0.01$.

Results

Lower (worse) smoking score was associated with lower (adverse) PAPP-A, higher (favorable) PlGF, and lower (favorable) Eng ($p<0.01$ for each). Higher PA score (healthier) was associated with lower (adverse) PAPP-A and lower (favorable) sFlt ($p<0.01$ for each). Higher PA was associated with lower APO occurrence ($p<0.05$).

Conclusions

Smoking exhibited unfavorable associations with PAPP-A, suggesting a potential mechanism for worse pregnancy outcomes in smokers. In contrast, lower Eng and higher PlGF in smokers may be related to the paradoxical protective effect of smoking against preeclampsia. While more PA was associated with lower risk of APO it was also related to lower PAPP-A. PAPP-A may be sensitive to PA through mechanisms independent of APO development.

Poster 120

An Innovative Hybrid Data mining model for imbalanced dataset of prognostication of Liver Disorder: A Naïve Bayes Classifier and K-Prototype Clustering Approach

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Liver disorders have recently become the deadliest disorder in many countries, with the number of patients increasing as a result of alcohol consumption, exposure to toxic gases, and ingestion of tainted foods and drugs. The data mining process is the most effective approach for detecting the disease early on. This study tries to predict and diagnose liver disease in its early stages. In this study, we used the Indian liver patient data set from the UCI machine learning repository. This data set contains the sex imbalance for which we applied both over-sampling and under-sampling strategies. For feature selection, we used Principal Component Analysis (PCA). In this research, we built 8 models from 4 experiments in R Studio with the required packages. These models are compared based on the performance factors, which include accuracy, sensitivity, specificity, and error rate. We made the Naïve Bayes model and a new innovative hybrid model combining k-prototype clustering and the Naïve Bayes Classifier (K-PNB) that detects liver disease in the early stages and diagnoses it in very little time. Thus, findings showed that the proposed hybrid model is the outperformer among them, with a classification accuracy of 99%, sensitivity of 99%, specificity of 99% and a low error rate of 0.0084%.

Poster 121

The Smart Cuff Project: Toward Multi-Parameter Hemodynamic Monitoring via a Single Convenient Device

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Patients in the critical care unit and those undergoing surgery require multi-parameter hemodynamic monitoring. Monitoring blood pressure, cardiac output (CO), and left ventricular ejection fraction (EF) is a great way to find hemodynamic instability and frequent low blood pressure, figure out the best treatment, and adjust interventions (like goal-directed therapy). Presently, however, these three hemodynamic variables require the use of some invasive, manual, or specialized instruments for measurement. The typical, non-invasive, and automated oscillometric arm cuff uses a population average algorithm to estimate blood pressure from the cuff pressure waveform, although this approach loses accuracy across the clinical range. In this work, we propose to develop an advanced arm cuff device with custom algorithms for accurate multiparameter hemodynamic monitoring. To achieve this, we created a custom device by integrating components from a home blood pressure (BP) monitor, including a micro-air pump, solenoid valve, and pressure transducer. The microcontroller was programmed to control the air pump and valve, enabling the desired cuff inflation and deflation patterns. We housed the device components in a 3D-printed encasing to ensure robust use in clinical settings. We checked the device functionality with a simulator and in pilot patients. The work involves building this arm cuff device to record cuff pressure waveforms, acquire patient data for algorithm training, and develop algorithms to compute BP, cardiac output (CO), and ejection fraction (EF) from the cuff pressure waveform. We will incorporate custom algorithms into the device, analyze the training data to refine or adapt previous physiologic algorithms, and investigate potentially superior machine learning algorithms for estimating these three hemodynamic variables. The final algorithms will be implemented on a real-time device, which will be validated against reliable reference measurements during clinical interventions.

Poster 68

Functional characterization of EPHA3 exon 4-5 duplication (EPHA3d4-5) in high-grade serous carcinoma progression, and recurrence

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Abstract

High-grade serous ovarian cancer (HGSC) accounts for 70-80% of ovarian cancer mortality, and overall survival has not been improved for decades. While standard therapy typically induces an initial response, most HGSC patients develop recurrent diseases following chemotherapy. The genetic aberrations that can be targeted to manage chemo-resistance remain ill understood. This suggests a desperate need to identity new therapeutic targets for the management of HGSC. Intragenic rearrangements (IGRs) leading to duplication or deletion of one or more exons have been sporadically reported to be cancer drivers. However, IGRs have not been rigorously studied in HGSC despite their potential significance as genetic drivers.

Our analysis of TCGA copy number data revealed that IGRs as a special type of genomic rearrangements may be far more frequent events than realized in HGSC. In addition, this analysis identified a duplication of exons 4-5 in the Eph-Like Receptor Tyrosine Kinase A3 (EPHA3) in 8.3% of HGSC tumors, which we termed EPHA3d4-5, and our recent data suggest that it may be far more frequent in recurrent tumors. Exon 4-5 duplicated EPHA3 transcript encode in an in-frame protein with an extra fibronectin type 3 domain, which we speculate could alter the function of EPHA3 protein. Indeed, specific knockdown of EPHA3d4-5 potently reduced the viability of the EPHA3d4-5 positive HGSC cell line, which is not observed in the EPHA3 wild-type cell line, suggesting that EPHA3d4-5 may drive cancer cell growth in HGSC. Furthermore, transcriptome sequencing of genetic perturbation and ectopic overexpression models revealed that EPHA3d45 appears to activate cell cycle, oncogenic Rho signaling, and MYC pathways, and suppresses apoptosis, P53, TGF-β and interferon signaling.

We thus hypothesize that EPHA3 exon 4-5 duplication may play a key role in promoting HGSC progression and recurrence and thus constitute a viable therapeutic target.

Poster 122

Single cell modeling of tumor-immune crosstalk in Age-related chronic inflammation in breast microenvironment promotes breast cancer in older women

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Background: Older women aged ≥70 years account for one-third of all breast cancer cases in the United States. We have limited understanding of the unique aspects of tumor microenvironment (TME) in older women with ER+ breast cancer. We hypothesize that specific inflammatory pathways or other significant drivers are involved in tumor-immune cell crosstalk in the TME of older women.

Methods: We analyzed bulk RNA-seq of ER+ breast cancer samples from 83 tumor or 85 tumor-adjacent normal tissue obtained from Pitt Biospecimen Core (PBC) and grouped them by chronical ages. Also, we obtained the publicly available scRNA-seq datasets of ER+ breast tumors (Younger, n=3; Mid-age, n=4; Older, n=3) from Wu et al. (PMID: 34493872) to characterize the TME and inflammatory differences between younger and older women with ER+ breast cancer.

Results: In bulk RNA-seq data analysis, we observed the expression of HSD17B7, an enzyme that converts estrogen from estrone (E1) to a more potent estrogen form, estradiol (E2), is positively correlated with estrogen pathway. scRNA-seq analyses showed that macrophages were the most transcriptomically distinct between younger and older women and the differentially expressed genes (DEGs) indicated the enrichment of inflammation-related pathways in older women with ER+ breast cancer, such as hedgehog signaling and TGF-β signaling. Interestingly, we identified an increased expression of immune checkpoint markers, such as CD274 (PD-L1), PDCD1LG2 (PD-L2), and M2-like macrophage markers, CD163 and MRC1 in macrophage of the mid-age and older women with ER+ breast cancer. We observed higher levels of CD206+ macrophages in both the tumor and stroma of older women with ER+ breast cancer.

Conclusion: This study provides insights into hormone disposition and the relationship between macrophages and cancer-cell derived inflammatory factors in older women with ER+ breast tumors. Our results provide evidence to support pharmacologically targeting paracrine regulator of tumor cell-macrophage interactions in the pro-tumorigenic older women breast TME.

Poster 123

Boltzmann ensembles of protein structures: Integration of EPR with weighted ensemble annealing

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Many proteins adopt more than one functional state. While EPR spectroscopy can detect alternate states by measuring distributions of long-range distances between spin labels within a protein, it cannot resolve the structures of these states at the atomic level. Molecular dynamics (MD) simulations can, in principle, generate structures that are consistent with the EPR distances; however, conformational transitions between alternate states are often on inaccessible timescales. Here we integrate a weighted ensemble annealing (WEA) strategy with an EPR distance distribution to efficiently sample the conformational ensemble of α-class glutathione S-transferase (hGSTA1-1) enzyme under equilibrium conditions.

Poster 38

Unraveling Visual Processing Dynamics in Adolescence: Insights from Stereotactic Electroencephalography (sEEG)

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This study presents the first stereotactic electroencephalography (sEEG) dataset examining visual processing in an adolescent cohort. Participants viewed images from different categories - faces, words, houses, and objects - while undergoing sEEG recordings. We characterized the temporal dynamics and high-frequency broadband (HFB) power modulations associated with visual processing in this unique population.

Our analyses focused on both low-level visual features and high-level semantic representations captured by the neural responses. By leveraging the exceptional spatial and temporal resolution of sEEG, we contrasted the engagement of the ventral visual pathway, known for its role in object recognition, with the dorsal pathway, implicated in spatial processing and action guidance.

Preliminary findings suggest distinct category-selective response patterns along the ventral stream. Temporally, we observed differential event-related potential (ERP) components in the ventral vs. dorsal pathways, indicating divergent neural dynamics underlying visual processing in these two streams.

The findings shed light on the developmental trajectories of visual processing streams and their specialization for different aspects of visual cognition during adolescence. This work provides novel insights into the neural underpinnings of visual perception in adolescents, bridging a crucial knowledge gap in the literature.

Our approach highlights the potential of sEEG to elucidate the fine-grained spatiotemporal dynamics of cognitive processes in pediatric populations, paving the way for a deeper understanding of brain maturation and its implications for visual information processing.

Poster 69

Galectin-3 plays a critical intracellular role in macrophages by promoting the repair, removal, and replacement of damaged lysosomes and protecting against atherosclerosis

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Galectin-3 (Gal3) is a β-galactoside-binding lectin predominantly known as a secreted inflammatory biomarker in cardiovascular disease, with significantly elevated circulating levels in patients with atherosclerosis and myocardial infarction. However, the molecular mechanisms of Galectin-3 action and whether its intracellular role contributes to atherogenesis remain unclear. Using several databases of bulk and single cell RNAseq, we first show that Gal3 transcripts are upregulated during plaque progression with particular abundance in the myeloid/macrophage lineage and foamy macrophages. Furthermore, Gal3 transcripts correlate significantly with increases in a network of lysosomal genes, suggesting a possible link between macrophage Gal3 and lysosomal function. Indeed, instigation of lysosome membrane damage in primary macrophages via cholesterol crystals and LLOMe triggers robust recruitment of Gal3 to lysosomes by sensing exposed carbohydrate moieties of proteins including Lamp1. Gal3 recruitment initiates a two-pronged lysosomal recovery program composed of either lysosomal repair involving the ESCRT complex or lysosomal removal and replacement involving autophagy/lysophagy and TFEB-mediated lysosomal biogenesis. Gal3-KO macrophages corroborate these findings displaying blunted ESCRT recruitment, diminished autophagy and TFEB activation, and resultant accumulation of damaged lysosomes. Gal3-deficiency also results in enhanced apoptosis and inflammasome/IL-1β activation upon triggering of lysosomal stress in macrophages. These findings are recapitulated in vivo, where Gal3-null bone marrow transplanted in atherogenic LDLR-null mice yields increased lesion size as well as altered plaque composition with macrophage accumulation, apoptosis, and necrotic core formation, characteristic features of the advanced plaque. Taken together, our data implicate unique and previously unrecognized protective function for intracellular Galectin-3 in macrophages and atherosclerosis which are distinct from its traditional role as an inflammatory biomarker in cardiovascular disease.

Poster 70

Landscape of intragenic rearrangements in triple-negative breast cancer reveals RUNX1 exon aberrations driving tumor immune evasion

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Triple-negative breast cancer (TNBC) is the deadliest breast cancer subtype, accounting for 10-20% of breast cancer morbidity. Chemotherapy remained the mainstay of intervention for TNBC due to the lack of well-defined genetic targets, and recent genomic sequencing studies have revealed a paucity of TNBC-specific mutations. In this project, our landscape study of genomic rearrangements in TNBC revealed that recurrent intragenic rearrangements that result in one or more exons being duplicated or deleted may constitute a major TNBC-specific genetic landscape that may contribute to its pathobiology. As proof of concept, we discovered novel intragenic rearrangements (IGRs) involving RUNX1, a proto-type cancer gene, which dictate an immune contexture in TNBC tumors that lack lymphocyte infiltrates. RUNX1 IGRs are preferentially detected in approximately ~7% of TNBC, which results in in-frame rearranged proteins that disrupt the RHD domain required for DNA binding and interaction with the CBFβ regulatory protein. RUNX1 is a master regulator that plays key roles in hematopoiesis, epithelial cytokine production, and induction of immune response. Our data suggest that RUNX1 rearrangements lead to potent repression of RUNX1 and NFκB target genes, resulting in upregulation of immunosuppressive cytokines such as CCL5 and repression of key proinflammatory cytokines such as CXCL10, suggesting its role in dictating tumor immune contexture. RUNX1 rearranged tumors are more aggressive showing larger tumor sizes, geographic necrosis, relative cold immune microenvironment that lack interferon γ signature and lymphocyte infiltration (especially CD8+ and CD4+ T cells), and a devastating clinical outcome. To date, this is the first report of somatic RUNX1 exon rearrangements in solid tumors, and the first study of their functions on regulating cancer immune landscape.

Poster 19

Hormone-Dependent Sex Differences in the Auditory Brainstem Response

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Females of multiple mammalian species (mice, rats, and humans) have higher auditory brainstem response (ABR) wave I amplitude than males. Wave I amplitude measures the recruitment and synchrony of spiral ganglion neurons (SGNs) in the cochlea, leading to better perception of auditory signals in background noise. There is interest in 17β-estradiol (E2) as a possible mediator of this sex difference since females have higher levels of circulating ovary-derived E2 than males. However, few studies have directly manipulated levels of gonad-derived sex hormones (E2 and androgens) to examine how they modulate SGNs, especially the effect of androgens in males. Additionally, no studies have examined the potential genetic effects of sex (i.e., Y- or X-linked genes) in hearing sensitivity.

To test the hypothesis that gonad-derived E2 and androgens mediate the sex difference in ABR wave I amplitude, we gonadectomized female and male C57BL/6J mice and compared wave I amplitude to sham littermates. Furthermore, using four-core genotypes (FCG) mice, we tested potential genetic effects of sex on wave I amplitude in addition to and in combination with hormone effects. In viable male FCG mice, the testis determining Sry gene has been de-coupled from the Y chromosome, and breeding these males with WT females allows for offspring of any combination of genetic (XX vs. XY) and gonadal sex (ovaries vs. testes). Thus, with FCG mice we tested main effects of sex hormones, sex chromosomes, or their interaction on ABR wave I amplitude.

Data from C57BL/6J mice show that the sex difference in wave I amplitude is eliminated following gonadectomy, due mainly to a reduction in wave I in females. FCG ABR data also show that sex hormones drive the sex difference in ABR wave I amplitude, as individuals with ovaries had higher ABR wave I than individuals with testes, regardless of genetic sex. In sum, the data indicate that the sex difference in ABR wave I is mediated by differences in sex hormones, mainly E2, with no detectable effect of genetic sex. This difference may enable females to better detect signal in noise.

Poster 71

A TBK1-independent primordial function of STING in lysosomal biogenesis

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The cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) innate immune signaling is activated in many pathophysiological conditions, leading to TBK1-dependent production of type I interferons in higher organisms. However, the primordial functions of STING independent of TBK1 are not well understood. Here, through unbiased proteomics and bioinformatics approaches, we identify lysosomal biogenesis as an unexpected function of STING. TFEB, an evolutionarily conserved regulator of lysosomal biogenesis and host defense, is activated by STING from multiple species. STING-mediated TFEB activation is independent of TBK1, but it does require STING trafficking and its conserved proton channel. GABARAP lipidation, stimulated by the channel of STING, is a key mechanism for STING-dependent TFEB activation. STING stimulates global upregulation of TFEB-target genes mediating lysosomal biogenesis and autophagy. TFEB signaling supports cell survival during chronic STING activation, a common condition in aging and age-related disease. These results reveal a primordial function of STING in the biogenesis of lysosomes, essential organelles in immunity and cellular stress resistance.

Poster 20

BET inhibition modulates the functional phenotype and plasticity of tumor associated macrophages and improve survival: Next frontier in overcoming immunotherapy resistance in ovarian cancer

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Purpose: Ovarian cancer (OC), with its high recurrence rates, poor patient prognosis and highest mortality rate urges need to develop immunotherapeutic strategies that can target both tumor cell and tumor microenvironment (TME). Tumor associated macrophage (TAMs) are prominent cells that contribute to immunosuppressive TME and therapy resistance in OC. Therefore, we investigated the role of Bromodomain and Extra-Terminal motif (BET) inhibitor (BETi) on TAM phenotype and consequently on TME and survival of OC models.

Experimental Design: We conducted a series of *in vitro* monoculture (qPCR, flow cytometry, and multiplex immunoassay), co-culture (qPCR, chipCytometry and multiplex immunoassay) of human OC cell lines as well as *in vivo* syngeneic ID8-VEGF OC model (qPCR, flow cytometry, and multiplex immunoassay), human blood sample (Nanostring-immune profiling) and human tumor biopsy sample (Gene Set Enrichment Analysis) experiments to determine the efficacy of BETi in OC. Statistical significance was determined through a two-tailed Student's t test. $p<0.05$ were considered statistically significant.

Results: Pre-clinical data from OC cell model revealed downregulation of CD47, CCL2, CSF-1 and CSF-1R genes at mRNA level and CD47, CCL2, CCR2 and IL-6 at protein level with BETi. Effective reprogramming of TAMs with BETi was evidenced by downregulation of M2-related markers, CD163, CD206 and CCL2 and upregulation of M1-related markers, iNOS, CD11b and CXCL10 along with longer survival in tumor-bearing mice. BET inhibition also resulted in the activation of STING marker, IFN-β and T-cell marker Granzyme B in CD57BL/6 mice consequently promoting innate and adaptive immune response.

Conclusions: Besides targeting inflammatory monocytes/macrophages and inhibiting the immunotolerance microenvironment and enhancing the anti-tumor immunotherapeutic effect via CCR2-CCL2 signaling, BETi also targets CD47 receptor by blocking CD47-SIRPα axis. This novel strategy could benefit OC patients by achieving clinical success in future.

Poster 72

A Spatial Transcriptomic "Google Map" Of Human Osteosarcoma Reveals Common Cell Clusters At The Primary Site And Pulmonary Metastasis

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Osteosarcoma (OS) is the most common primary malignant bone tumor with poor prognosis. As the survival rate for patients with metastases remains at only 20%, new research approaches are needed to better understand the mechanisms of progression of metastases. Through spatial transcriptomic analysis of three pairs of matching primary and lung metastases (LM) of osteosarcoma obtained from three patients, we aimed to describe the identity, localization, transcriptomic profiles, and developmental interplay of tumor and non-tumor cells. Upon collection, samples were processed with 10x Genomics Visium Spatial Gene Expression for FFPE samples®, according to the provided protocol. The unbiased cluster analysis of all samples identified 12 major clusters. Boundaries of the clusters overlapped with the edges of the histological regions identified by the pathologist. Three clusters of malignant osteosarcoma cells (MOCs) presenting an osteogenic-like phenotype were detected in the tumoral areas. Pathway analysis revealed a significant decrease in apoptosis and cell death signaling along with a pronounced increase of cell invasion and migration signaling. In addition, a comparison between MOCs of the primary OS cells vs MOCs of the LM revealed that MOCs of the metastases express higher levels of genes typically present in the lung (SFTPB, SFTPC) which are absent in primary OS, suggesting that these genes may be responsible for the metastases' onset and development within the lungs. Immune cell clusters were found mainly in the LM localized at the tumor-lung interface in all patients and low expression of CD8, CTLA4, and PD-1 was detected in all samples, indicating a limited T-cell infiltration within the tissues, which may explain the limited efficacy observed in OS clinical trials targeting the PD-1/PD-L1 pathway. This study presents a spatial transcriptomic analysis of primary and metastatic lesions of human OS, in the form of a consultable "Google Map", offering the opportunity to retrieve useful information about the localization, characteristics, and developmental dynamics of human OS.

Poster 21

Speech and melody processing in the same hemisphere after pediatric cortical resection: an fMRI investigation

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We aim to characterize plasticity in perisylvian language cortex following the resection of perisylvian regions in the opposite hemisphere during childhood. In adults, left hemisphere (LH) stroke causes debilitating language impairments, and reorganization of language abilities to the preserved right hemisphere (RH) is limited, whereas reorganization is more successful in young children. We will carry out functional mapping of the preserved perisylvian regions after cortical resection in childhood to better understand the competitive dynamics of neuroplasticity that govern how certain cognitive functions that become atypically lateralized after injury are organized alongside functions that remain typically lateralized.

We will examine speech and melody processing, which are ordinarily lateralized to the LH and RH, respectively. Individuals with cortical resections (n=13 LH and n=13 RH) for the management of drug-resistant epilepsy (DRE) will be recruited, as well as age-matched controls. Aim 1 is across-sectional comparison of the language-related functional MRI (fMRI) activation profiles in DRE participants and controls. Aim 2 is a longitudinal fMRI study of post-surgical DRE participants to evaluate plasticity in the preserved hemisphere as functional networks stabilize during recovery. Age-matched controls will also be scanned longitudinally. We are using a modified version of a task that has shown robust effects in healthy adults, which involves 10 naturalistic a cappella song stimuli (10 sentences x 10 melodies). Each participant's fMRI data will be analyzed using multivariate support-vector regression to classify brain areas as speech-selective, melody-selective, or non-selective. This classification will be performed in both hemispheres for controls, and in the preserved hemisphere in DRE participants. In DRE participants' preserved hemispheres, we will investigate (1) deviations from the typical organization of speech and melody processing in the LH and RH, and (2) longitudinal changes in speech- and melody-selective areas after surgery.

Poster 124

The Paraquinone + H2 → Hydroquinone Reaction: A Challenge for Diffusion Monte Carlo Calculations

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In this study, we explore the application of both coupled cluster singles plus doubles with perturbative triples (CCSD(T)) and diffusion Monte Carlo (DMC) methods to accurately compute the energy of the paraquinone + H2 → hydroquinone reaction. Initially, employing single Slater determinant trial wave functions in DMC calculations, we find a significant discrepancy of nearly 4 kcal/mol compared to CCSD(T) results, underscoring the challenge of achieving precise energetic predictions using DMC.

However, by transitioning to multideterminant trial wave functions in DMC calculations, particularly those capturing static correlation, we observe a notable improvement. The discrepancy with CCSD(T) values is reduced by about half, indicating the importance of incorporatingmultiple determinants to better represent the electronic structure,especially in addressing nodal surface errors inherent in DMC methodologies. This success underscores the significance of apturing static correlation, crucial for accurately describing nodal surfaces, and its substantial impact on the precision of DMC predictions.

Understanding and refining these methodologies not only contributes to our comprehension of chemical reactions but also holds potential implications for enhancing the reliability of datasets used in machine learning applications. By elucidating the intricacies of balancing nodal surface errors and accurately representing electronic structures, these insights pave the way for developing more robust data sets crucial for training machine learning models in chemical sciences.

Poster 73

Functional dissection of Atoh7 cis-regulatory elements in genesis of retinal ganglion cells

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Atoh7 plays a critical role in the specification, differentiation, and development of retinal ganglion cells (RGCs) and optic nerve. It is transiently expressed in retinal precursor cells, yet the specific functions of its cis-regulatory elements in governing RGC development remain unclear. In humans, the loss of a distal regulatory element near Atoh7 is linked to optic nerve aplasia and consequent loss of vision. This study investigated the necessity of Atoh7 enhancer elements in mouse retinal development. Two mouse models were generated with knockout mutations targeting distal/remote enhancer (Atoh7-RE), region and entire enhancer landscape (Atoh7-EN) of Atoh7. Remarkably, targeting the elimination of a distal enhancer (Atoh7-RE) did not affect RGC formation but caused dysregulation of axonogenesis genes and disrupted RGC axon innervation to the brain. Moreover, our transcriptomic analysis at E14.5 revealed significant downregulation of Atoh7 expression, although its downstream gene regulatory network remained robustly expressed. Furthermore, single-cell RNA sequencing (Sc-RNA-seq) analysis indicated disproportional effects on Atoh7 expression in specific cell clusters, with notable expression persisting in neurogenic precursor cells. In contrast, another mutation targeting the entire Atoh7 enhancer landscape (Atoh7-EN-KO) resulted in complete loss of RGCs, optic nerve hypoplasia, impaired retinal function, and vascular alterations, primarily due to the significant downregulation of Atoh7 and its crucial gene regulatory network essential for RGC genesis. Together our findings highlight the essential role of the Atoh7 enhancer landscape in RGC development and axongenesis, revealing differential requirements for Atoh7 enhancer elements between mice and humans.

Poster 125

Investigation towards a cuff-less, non-invasive wearable finger ring for blood pressure measurement

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Measuring blood pressure frequently using a wearable device connected with smartphone allows for continual monitoring and improved hypertension management. However, current arm cuff BP measuring instruments are less suitable for certain individuals, such as morbidly obese people, and their inability to facilitate anytime, anywhere monitoring of BP. We have developed a novel cuff-less, non-invasive finger ring for blood pressure measurement, integrating a force sensor, LED, photodetector, and accelerometer. The ring estimates blood pressure via an oscillometric hand lowering method. The ring can be tightened to apply a different amount of pressures on the finger, enabling the measurement of external pressure. By occluding the arteries and then slowly lowering the hand from high to low for changing the hydrostatic force, Photoplethysmogramic (PPG) oscillations are obtained. A customized algorithm is used to create an oscillogram and estimate blood pressure accurately.

To validate the effectiveness of the finger ring device, a prospective study was conducted with human subjects (n=10) to assess usability and accuracy compared to a standard automatic cuff-based device. Participants wearing rings were trained to occlude their finger arteries and perform the hand lowering technique within 30 seconds. The results revealed minimal bias and precision errors in the measurements of systolic and diastolic blood pressure, with correlations of 0.95 and 0.89, respectively, with respect to the reference arm cuff measurements.

A wired version of the ring has been presented here, and we are currently developing a wireless iteration featuring a flexible PCB encased within the ring. This version will incorporate a convenient tightening mechanism and a customized force sensor. This technology has the potential to revolutionize blood pressure monitoring, offering a convenient and reliable option for ubiquitous blood pressure measurement.

Poster 22

The zinc finger of DNA Ligase 3 is an autoinhibitory domain and binds to nucleosomes via an arginine anchor

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DNA ligation of DNA single strand breaks (SSB) is critical for maintaining genome integrity during DNA replication and repair. X-ray cross complementing protein 1 (XRCC1) forms an important scaffolding complex with DNA Ligase 3 (LIG3α) during base excision repair (BER). To measure the dynamics of LIG3α-XRCC1 interactions with SSB in naked DNA or in chromatin, we used a real time single-molecule approach to visualize Halo-tagged LIG3α with XRCC1-YFP, from nuclear extracts. On naked DNA, LIG3α and XRCC1 showed strong association with ligatable nicks, with lifetimes of 5.4 s and 6.9 s, respectively, with 17.3% of the events co-localized. Domain analysis suggests that the N-terminus zinc finger acts as an autoinhibitory domain for stable LIG3α interactions. LIG3α and XRCC1 were found to bind to an undamaged 601 nucleosome core particle (NCP) with lifetimes of 68 s and 33.1 s, respectively and colocalization frequency was 35.2%. Deletion analysis indicated that the Zinc finger of LIG3 α was responsible for nucleosome binding, and strikingly replacement of two key arginine residues in the zinc finger domain with alanine abolishes the nucleosome binding.

Poster 105

Assessing Mobile Device Proficiency for a Smart Home Intervention in Individuals with Complex Disabilities

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Objective: To assess mobile device skills as part of the initial assessment of a smart home technology intervention for individuals with complex disabilities.

Design: This case series study involved participants completing a short version of the Mobile Device Proficiency Questionnaire (MDPQ-16) and participating in an observational assessment of mobile device skills. The assessment included five tasks and observational guidelines on device positioning, manual and voice access, and cognitive aspects.

Setting: Participants' homes.

Participants: A convenience sample of 13 individuals who use a power wheelchair and have difficulty independently controlling or accessing their environment.

Main Outcome Measures: MDPQ-16 and mobile device skill performance.

Results: Eleven out of 13 participants completed the MDPQ-16, with an average score of 31±9 (range: 16-40, max 40 for top proficiency). Twelve completed the mobile device assessment. Five used Android and eight used iOS. Five used voice input, four used touch, and two used both methods. Five participants (MDPQ range 36-40) had no noted difficulties during mobile device assessment. The other 8 (MDPQ range 16-39) exhibited diverse device skill performance stemming from device mounting and positioning challenges, motor impairments affecting touch gesture and/or typing precision, limited familiarity with the accessibility features, insufficient digital literacy, and outdated devices lacking necessary features.

Conclusions: Relying solely on the MDPQ-16 yielded inadequate insight into participants' mobile device skills. The observation-based mobile device assessment revealed specific areas of knowledge or functional deficits, enabling clinicians to tailor necessary skill training and adaptation recommendations. The MDPQ-16 is an inadequate assessment to use alone when understanding participants' mobile device skills but should be used in conjunction with an observation-based mobile device assessment to ensure foundational mobile device proficiency is achieved prior to smart technology training.

Poster 74

To the synapse and beyond: characterizing the regulatory mechanisms for α5 $GABA_A$ receptor distribution in hippocampal neurons

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In the adult brain, GABA type A receptors ($GABA_ARs$) generate fast inhibitory signals to dampen and control neuronal activity at the network and cellular levels. $GABA_AR$ function and pharmacology depends on subunit composition and arrangement, which is regulated by the spatial, temporal, and subcellular expression pattern of the 19 $GABA_AR$ subunits. α5 subunit-containing $GABA_ARs$ (α5 $GABA_ARs$) are enriched in the hippocampus and play a key role in neuronal development, synaptic plasticity, and cognitive function. Proper α5 $GABA_AR$ surface distribution and activity-dependent reorganization are critical for these roles; however, the regulatory mechanisms are poorly defined. α1-3 and α5 $GABA_ARs$ exhibit receptor clustering at synaptic sites due to direct interactions with the inhibitory postsynaptic scaffold gephyrin. Unlike other $GABA_ARs$, α5 $GABA_ARs$ also form receptor clusters at extrasynaptic sites due to direct interactions with the actin-binding protein radixin. While the gephyrin binding domain (GBD) is known, the radixin binding domain (RBD) remains elusive. Surprisingly, the RBD is reported to exist in a region of high homology between all $GABA_AR$ α subunits (AA 342-357). Conversely, the GBD exists in a region of much lower homology (AA 370-385). Our antibody-based proximity ligation and immunoprecipitation experiments assessing α5/radixin interaction in primary hippocampal neurons suggest that the RBD exists in an alternate region. We propose that this region, which overlaps with the GBD and contains two α5 phospho-sites – S374 and S406, contains a novel RBD that imparts subunit specificity. Further, we hypothesize that phosphorylation of S374 acts as a "switch" to control α5 $GABA_AR$ association with radixin vs gephyrin scaffolds, as phosphorylation is a key regulator of other $GABA_AR$/scaffold interactions. Here we will define the RBD, identify the role of phospho-dependent regulation of α5 $GABA_AR$ in receptor/scaffold interactions, and assess the impact of impaired radixin binding on neuronal development and inhibitory transmission.

Poster 39

Assessing Pain Severity in Sickle Cell Disease Using Animations and a Graphical Body Image

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Adults with sickle cell disease (SCD) frequently seek medical care due to pain. Accurate pain assessment is critical to determine treatment; however, current approaches for assessing SCD pain are inadequate. Painimation helps patients communicate pain quality, intensity, and location using abstract animations and a paintable body image. Painimation has been validated in a general population with chronic pain, but there is limited data in SCD. This study replicates and extends prior findings by determining whether pain animations and body image data are associated with pain outcomes in a large cohort of adults with SCD.

This study is a secondary analysis of baseline data from a randomized controlled trial in adults with SCD. Participants completed questionnaires and tracked their pain and mood daily via a mobile app. Participants were categorized by animations selected and proportion of the body image painted. Baseline characteristics and demographics were compared between groups, and multivariable regressions were used to estimate covariate-adjusted associations with pain-related outcomes and opioid misuse.

The trial enrolled 359 adults, mean age 36.3 (SD = 10.5), 66% female, 93% Black race. The "Shooting" painimation and greater body image scores were associated with all outcomes in univariate analyses except for the proportion of "happy" mood days and anxiety scores. After controlling for covariates, the shooting animations were independently associated with greater daily pain intensity, and greater body image score was associated with daily pain intensity, as well as pain severity, frequency, and interference.

The shooting animation and body image measures were associated with more severe pain outcomes. This study demonstrates that animations and body image data can be used to assess pain severity in SCD, more objectively than the 0-10 numeric visual analogue scale. Future studies should explore whether pain location and animation selected are associated with pain etiology and determine whether this approach can differentiate different types of pain in SCD.

Poster 106

PLASMA GFAP IS ASSOCIATED WITH WHITE MATTER ABNORMALITIES IN THE ALZHEIMER'S DISEASE CONTINUUM

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Objective: Investigate the association of plasma glial fibrillary acidic protein (GFAP) with brain volume atrophy in the context of amyloid beta (Aβ) and cerebrovascular burden as determined by white matter hyperintensities (WMH) in Alzheimer's disease (AD).

Methods: Our study involved 597 Aβ- and Aβ+ cognitively impaired individuals from a memory clinical cohort (BICWALZ, South Korea). Plasma GFAP levels were compared between individuals across the Fazekas scale (Fz;1-3), a clinical scale used to quantify the amount of WMH. We employed ANCOVA followed by Tukey Test to verify differences between groups. Linear regressions, accounting for age and sex, were used to estimate the associations.

Results: GFAP levels are higher in individuals with an Fz score of 2-3 compared to Fz 1 in both Aβ- (P=0.0006) and Aβ+ (P=0.0011) populations. However, GFAP was only associated with brain atrophy in the Aβ+ population during the Fz 1 stage (~GFAP: β = -0.2367, P = 0.0229; GFAP*Fz: β= 0.3366, P = 0.0119). Further analyses for gray and white matter volumes individually showed GFAP being only associated with white matter atrophy in the Aβ+ population during the Fz 1 stage (~GFAP: β = -0.2412, P = 0.0201; GFAP*Fz: β= 0.3718, P = 0.0054).

Conclusion: Our findings suggest that in the context of concomitant Aβ and WMH burden, GFAP with white matter degeneration. Despite lower GFAP levels in Fz 1 compared to Fz 2-3, reactive astrogliosis may be crucial in white matter atrophy in early WMH burden and Aβ+ individuals. We suggest that therapies targeting astrogliosis in early WMH/white matter lesion stages could potentially alleviate cognitive decline in mixed AD-vascular dementia pathology.

Poster 40

Using time-driven activity-based costing to identify value improvement opportunities in Complex Rehabilitation Technology evaluations

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The UPMC Rehabilitation Institute Center for Assistive Technology (CAT) seating and mobility therapists aim for a 75% productivity rate, scheduling up to 4 patients daily. However, the 1.5-hour initial evaluation time for Complex Rehabilitation Technology (CRT), such as manual or powered wheelchairs, often proves insufficient for comprehensive assessments (patient education, mat assessment, molding, equipment trials, etc.) due to the patients varying needs. This often leads therapists to exceed the allotted time. This common occurrence suggests that an increase in evaluation times can resolve this issue yet interferes with meeting the productivity standards at CAT as it decreases the number of patients scheduled daily. Additionally, these therapists work with a medically complex population that also demands non-reimbursable time (i.e., writing letters of medical necessity, appeals, phone calls, follow-ups, etc.), forcing them to work additional hours outside clinic time. As a result of these two scenarios, therapists at CAT often experience a discrepancy between decreased productivity standards and increased workload during their week.

The objective of this study was to identify the total amount of time a therapist takes to complete all reimbursable and non-reimbursable tasks that a standard evaluation for a manual or powered wheelchair requires for a single patient. This study used a time-driven activity-based costing approach to analyze all therapist activities involved. As part of this ongoing study, it is anticipated to justify why therapists at traditional outpatient therapy settings with similar productivity standards often handle a higher patient load. Findings may support an adjustment in scheduling to allow for more thorough evaluations of CRT, impacting productivity standards, policy development, and revenue generation in similar clinics. Additionally, increasing time directly affects service delivery aspect of CRT by improving end-user, clinician, insurer, and supplier collaboration by providing a mobility device that best fits their needs.

Poster 41

Introducing the Educational Board Game for Community-Dwelling Mobility Devices Users and Their Caregivers

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The utilization of mobility devices in the US is on the rise, yet training for their proper use primarily occurs within clinical settings.

The Educational Board Game (EBG) is a simulation-based learning tool developed to promote safe and independent transportation for mobility device (e.g., wheelchair, scooter) users and their caregivers. As players navigate toward their destination on the game board, they engage in discussions about scenarios involving transportation barriers within the community, selecting the best response from four options. This study aimed to assess the initial validity and reliability of the 50 scenarios of the EBG.

In this cross-sectional study, individuals aged 18 or older using a mobility device as their primary means of transportation at least once a week, or their caregivers aged 18 or older travelling with mobility device users at least once a week, were included. Content validity was evaluated by measuring participants' agreement on the realism of the scenarios using a 5-point Likert scale. Item-level Content Validity Indices (I-CVIs) and Content Validity Ratios (CVRs) were calculated to assess the content validity of the 50 scenarios. Scenarios with I-CVIs of 0.78 or higher, in addition to CVRs meeting the critical level of agreement based on the number of respondents, were considered as acceptable level of content validity. Inter-rater reliability was assessed using the Intraclass Correlation Coefficient (ICC). ICC between 0.5 - 0.75 was considered as a moderate level of reliability.

Data from 24 participants, including 4 caregivers, were analyzed (Mean age: 59.4±15.1; Female: 32.1%). Five scenarios out of 50 did not meet an acceptable level of content validity. The overall ICC was 0.73 (95% Confidence Interval: 0.609; 0.826), indicating a moderate level of inter-rater reliability.

Consider revising or removing five scenarios and adding new ones that reflect community life events. Using the EBG can effectively enhance the safety and independence of mobility device users in transportation, whether in community or clinical settings.

Poster 75

Dissecting cause and effect of immune related Adverse Events in a novel mouse model.

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Immunotherapies targeting the inhibitory receptors PD-1 & CTLA-4 have brought incredible success in cancer treatment. However, ~90% & ~70% of patients receiving anti-CTLA4 or anti-PD1 develop immune-related adverse events (irAEs). Additionally, the failure of treatment strategies to mitigate serious irAE symptoms while maintaining immunotherapy efficacy results in abrupt termination of immunotherapy and toxic fatalities. One major obstacle in this endeavor has been the poor understanding of immunopathology of irAEs due to lack of a preclinical mouse model. Thus, we sought to develop a mouse model that faithfully recapitulates the clinical phenotypes of cancer patient irAEs via diet mediated perturbation of gut microbiome. We subjected B6 mice consuming either regular diet (RD) or western diet (WD) to 3 weeks of anti-PD1 treatment. Mice fed WD developed several immune toxicities including inflammation of colon, skin, pancreas, small/large intestine, & orofacial region upon receiving anti-PD1, while mice fed RD developed no symptoms. The onset of irAE symptoms was directly proportional to length of WD exposure before anti-PD1 administration. In colon, irAE was associated with significantly increased infiltration of $CD4^+$ T cells & dendritic cells and reduction of suppressive Tregs in epithelial layer. Taken together, we have generated a first of its kind irAEs mouse model which is simple yet effective in replicating the clinical symptoms of systemic irAEs in cancer patients.

Poster 76

Fatty acid metabolizing enzyme CYP4F11: substrate channel investigation in free and membrane bound states

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Lung cancer is the leading cause of cancer-related deaths worldwide. The cytochrome P450 (CYP) isoform CYP4F11 is a member of CYP enzymes and is considerably overexpressed in patients with lung squamous cell carcinoma. CYP4F11 is a fatty acid ω-hydroxylase that catalyzes the generation of the lipid mediator 20-hydroxyeicosatetraenoic acid (20-HETE) from arachidonic acid. 20-HETE participates in cancer cell proliferation and migration. The inhibition of 20-HETE-generating cytochrome P450 enzymes has been implicated as a novel cancer therapy. Mammalian CYP4F subfamily of enzymes are endoplasmic reticulum membrane-attached proteins involved in many biotransformation processes. Membrane binding affects the enzyme behavior and may alter the opening of access/egress channels and substrate/metabolite transport into/from the buried CYP's active site as well as modify interactions with redox partners driven by electrostatic interactions. To better understand these effects, we performed molecular dynamics simulations of CYP4F11 both in its free and membrane bound states. We embedded CYP4F11 into a POPC lipid bilayer and ran three replicates of AMBER molecular dynamics using the ff15ipq force field. Then the pocket volume of the substrate channel in the unbound (apo) state and in complex with arachidonic acid (AA) was investigated. The pocket volume of the CYP4F11 substrate channel in the case of apo-free, and apo-bilayer embedded states shows the values of 659.05 ± 61.45 and 947.47 ± 77.76, respectively which suggests a profound increase of the substrate channel volume in the embedded state. Also, the pocket volume shows higher values in the case of AA-bilayer embedded state compared to the free state with the values of 1040.34 ± 57.11 and 924.83 ± 54.33, respectively. The interaction pattern of AA in the free and membrane embedded states changes mostly in the case of hydrogen bond interactions of terminal carboxylic acid oxygens. In the AA free state, the carboxylic oxygens make hydrogen bonds with Gln237 and Val67. However, in the AA-bilayer embedded state, differences in the AA conformation makes the amino acid residues Asn423, Leu77, and Ile100 accessible for the hydrogen bond interaction. This conformation change is mostly due to the steric hindrance of the membrane, making the AA tail orient towards the inside of the CYP4F11 substrate channel. In summary, the CYP4F11 substrate channel shows higher pocket volume values when embedded in the bilayer and makes different enzyme residues accessible for hydrogen bond interaction. These studies may point the way to designing selectivity and physiologically relevant inhibitors of CYP4F11.

Poster 23

Epitranscriptomic signaling in oral mucosal defenses against *Candida albicans*

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Fungal infections are a major global threat, yet to date there are no vaccines to any pathogenic fungi. *Candida albicans* (*C. albicans*) is considered a WHO priority pathogen due to its capacity to cause severe morbidity and mortality in millions of individuals worldwide. Oropharyngeal candidiasis (OPC, oral thrush) is rare in immunocompetent individuals, but can cause severe infections in susceptible hosts, associated in some cases with esophageal cancer, malnutrition and failure to thrive in infants. However, mechanisms of oral mucosal immunity remain poorly defined, especially as compared to better-studied mucosal tissues such as gut and lung. Oral epithelial cells (OECs) are the first cells to encounter *C. albicans* during infection, and these cells orchestrate a complex array of molecular mucosal innate defenses that prevent disease development, in part by direct antifungal actions but also by activating pathways that drive protective Th17/IL-17-based immunity. There have been extensive studies characterizing the transcriptional mechanisms in OECs that are induced upon sensing *C. albicans*. However, studies in recent years have shed light on a less-appreciated mechanism of gene activation through "epitranscriptomic" pathways, including RNA modification. To date, almost no studies have sought to understand how changes in the epitranscriptome influences OEC-dependent immunity to fungal infection. Increasing evidence has shown that the N6-methyladenosine (m^6A) modification of mRNA is associated with regulation of the innate immune response to viral and bacterial infection. Here, we demonstrate a role for m^6A modification in driving epithelial immune responses to *C. albicans* infection in OECs. Upon targeting of core m^6A enzymes and "reader" proteins, we have uncovered significant reprogramming of *C. albicans* host defense genes. Ultimately, defining the molecular underpinnings of host immunity to infection may pave the way for novel targets to modulate barrier immune responses against fungal pathogens.

Poster 107

The effect of microglial activation on brain atrophy across the Alzheimer's disease continuum

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Objective: To investigate the influence of microglial activation on cross-sectional and longitudinal brain atrophy, and future cognitive decline in Alzheimer's disease continuum.

Methods: We investigated 124 individuals from the TRIAD cohort with measures of brain voxel-based morphometry, microglial activation (MA) [^{11}C]PBR28 PET, tau [^{18}F]MK6240 PET, Aβ [^{18}F]AZD4694 PET, and CDR sum of boxes. We performed regional-wise partial correlation analysis to investigate the association between MA and brain density. We calculated the rate of change (RoC) for the longitudinal analyses. We applied the principal component analysis for MA and brain density RoC in the longitudinal analysis.

Results: Regional-wise analysis reveals a mix of positive and negative associations for cognitively unimpaired (CU) individuals. When divided by amyloid-β (Aβ) status, Aβ- individuals exhibit only positive associations. In contrast, Aβ+ individuals demonstrate only negative associations. In CU Aβ+ individuals, we observed a synergistic association between MA and tau pathology, leading to future brain atrophy and cognitive decline. Next, we performed the same analysis for the CI Aβ+ individuals. In early Braak stages, we found only positive associations between MA and brain atrophy, while in late Braak stages, only negative associations were apparent. The interaction of MA and tau pathology synergistically predicted longitudinal brain atrophy and cognitive decline in CI individuals.

Conclusion: Our study found waves of positive and negative associations between MA and brain density modulated by Aβ in the early stages of the disease and by tau in the later stages. Furthermore, the interaction of MA with tau seems decisive for longitudinal brain atrophy and future cognitive decline in both the early and late stages of the disease.

Poster 77

Prospective Associations of Midlife C3, C4 and Their Changes Since Midlife with Future Cognitive Performance: The Study of Women's Health Across the Nation (SWAN) HDL Ancillary Study

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Objective: Almost two-thirds of Americans over 65 with Alzheimer's disease (AD) are women. The menopause transition is characterized by a systemic inflammatory state which increases atherosclerosis risk predisposing to dementia. Complement system was inappropriately activated in the brain of AD patients, which may contribute to local inflammation correlated with cognitive dysfunction. Complement factor 3 (C3) and 4 (C4) are the most clinically used complement components. Increased serum C3 and the presence of C4 null genes producing less C4 are risk factors of cardiovascular disease (CVD), indicating the necessity of analyzing the ratio of C3 and C4. In addition, women experienced greater increase in C3 than C4 over the menopause transition. Therefore, we aimed to assess the associations of midlife C3, C4, and C3/C4 ratio and their changes since midlife with future cognitive function in women. We hypothesized that higher or increase in C3 and C3/C4 ratio, and C4 may be negatively and positively associated with future cognitive performance, respectively.

Design: Repeated measures of serum C3, C4, and measures of working memory (digit span backward test), processing speed (symbol digit modality test), episodic memory immediate and delayed recall (East Boston memory test) were evaluated among midlife women traversing menopause. Mixed effect models were applied to assess the associations.

Results: We included 305 women (706 observations) with mean age of 51 (SD=3) and 72% of them being pre- or perimenopausal at baseline. C3 and C4 were measured a median of 1.07 (Q1: 0.94, Q3: 1.81) years before cognitive performance assessment. In final models, higher C4 measured at midlife (baseline) was associated with better future episodic memory levels, and higher midlife C3/C4 ratio was associated with worse future episodic memory (**Table**). Changes in the complement factors were not associated with cognitive measures.

Conclusion: Among women, higher midlife C4 and lower midlife C3/C4 ratio, but not changes in C4 or C3/C4 ratio since midlife, were associated with better future episodic memory levels. Midlife complement protein levels could be early markers of cognitive impairment known to be linked with CVD.

Poster 78

Evaluating the therapeutic potential of the small molecule ATR inhibitor BAY 1895344 and radiotherapy in head and neck cancer

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Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cancer worldwide. For locally advanced human papillomavirus (HPV)-negative HNSCC, recurrence rates approach 50% despite aggressive standard treatments like chemoradiation or surgery with adjuvant radiation therapy (RT) +/- chemotherapy. There is a critical need for more effective therapeutics to improve outcomes for HNSCC patients. Ataxia telangiectasia and Rad 3-related (ATR) protein kinase is a potential therapeutic target for improving RT response. We hypothesize that inhibiting ATR radiosensitizes tumors by bypassing DNA repair and forcing HNSCC cells with unrepaired DNA damage into mitosis, leading to mitotic catastrophe (MC). We used ATR inhibitor BAY 1895344 (BAY) to study this in preclinical models. We confirmed that BAY inhibits ATR via Western blot analysis of downstream CHK1 phosphorylation in RT versus RT+BAY treated cells. In clonogenic assays, cells treated with BAY and RT demonstrated radiosensitization compared to vehicle ($p<0.05$, unpaired t-test). Cell cycle analysis confirmed that BAY significantly abrogated RT-induced G2/M cell cycle arrest ($p<0.01$, unpaired t-test). Cells treated with BAY before RT vs RT or BAY alone exhibited increased co-staining for yH2AX and pHH3, indicative of MC. In a radioresistant MOC2 syngeneic mouse model, tumors were induced in the hind limb of C57BL/6J mice. Mice were randomized (n=9-11/group) to receive vehicle, BAY (40mg/kg), vehicle + RT (8Gy x 3), or BAY + RT. Fractionated RT was administered every 4 days. BAY was given orally twice on the day of RT (AM/PM) and once the morning after RT. Median survival was significantly increased in treated groups: 39.5d for BAY + RT vs. 14d for vehicle ($p=0.0002$, Log-rank test), 22d for BAY ($p=0.0003$), and 23d for vehicle + RT ($p=0.0007$). Thus, the ATR inhibitor BAY 1895344 radiosensitizes HNSCC *in vitro* and increases survival combined with RT *in vivo,* indicating therapeutic potential for targeting ATR in HNSCC and warranting a phase I clinical trial (NCT04576091).

Poster 79

Matrix-Bound Nanovesicles Mitigate Neoplastic Esophageal Cell Phenotype

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Esophageal cancer stands as the sixth most prevalent cause of cancer-related mortality worldwide. Moreover, the incidence of esophageal adenocarcinoma (EAC) is increasing at an alarming pace. The current standard of care, esophagectomy, is associated with high morbidity, post-surgical complications, and decreased quality of life, underscoring the critical need for innovative therapeutic approaches. Biologic scaffolds, composed of allogeneic or xenogeneic extracellular matrix (ECM), have emerged as promising solutions for various clinical applications, including esophageal reconstruction. Additionally, matrix-bound nanovesicles (MBV), integral components of ECM, have demonstrated remarkable potential in immunomodulation, particularly in activating a pro-remodeling macrophage phenotype.

Recent studies from our laboratory have shown the impact of ECM bioscaffolds, particularly their degradation products, on both metaplastic and neoplastic esophageal cells, resulting in mucosal remodeling in rodent and canine models. In this study, we aimed to explore the potential role of MBV, beyond its immunomodulatory effects, hypothesizing its direct influence on the neoplastic phenotype of esophageal cells.

Our findings reveal that MBV derived from porcine urinary bladder (UBM-MBV) can be internalized by epithelial cells, leading to the downregulation of the neoplastic phenotype. Interestingly, while normal esophageal cells maintain their physiological characteristics in culture, neoplastic esophageal cells exhibit a pronounced cytotoxic response to MBV, partially dependent on caspase-3 activity. These observations suggest a cytotoxic potential of MBV in esophageal cancer cells, offering pivotal insights for advancing next-generation therapeutic strategies in the treatment of esophageal diseases.

Poster 24

Intraoperative Molecular Imaging of Neuroblastoma with DTPA[In-111]-dinutuximab-IR800

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Introduction: Surgical resection is a critical component of neuroblastoma treatment, but distinguishing tumor from neurovascular structures and identifying remote disease remains challenging. We designed a novel intraoperative molecular imaging (IMI) tracer, DTPA[In-111]-dinutuximab-IR800, which targets GD2 (overexpressed in 99% of neuroblastoma) for detection of gamma decay from DTPA[In-111] and fluorescence visualization of IRDye800CW. We hypothesize that binding and accumulation is dependent on the number of IR800 per tracer.

Methods: DTPA-dinutuximab-IR800 was synthesized with a range of IR800/tracer ratios, and ELISA was performed. SK-N-BE(2) orthotopic neuroblastoma xenografts were established in nude mice. Four days after injection of DTPA[In-111]-dinutuximab-IR800 (n=4-6 per group), biodistribution was performed and intraoperative tool use was assessed.

Results: While the IR800/tracer ratio did not impact tracer accumulation in neuroblastomas (7.0-10.8 %ID/g), increased ratios decreased binding affinity (Kd 5.2, 18.63, and 75.79 for 1.55, 2.70, and 4.37 IR800/tracer, respectively) and increased liver accumulation (2.09, 5.55, 7.42, and 8.81 % ID/g tissue and 8.6, 10.1, 13.2, and 14.3 fluorescence units, for 1.18, 1.72, 2.14, and 2.71 IR800/tracer, respectively). Intraoperative tools (Neoprobe and SPY-PHI) were able to detect and visualize tracer accumulation in tumors.

Conclusions: While the IR800/tracer ratio did not affect tracer accumulation in tumor, higher ratios decreased GD2 binding and increased non-specific liver accumulation, impairing the signal-to-noise ratio. Furthermore, the tracer enabled detection with common intraoperative tools (Neoprobe and SPY-PHI).

Poster 25

Influence of different cutoff techniques in determining tau positivity in Flortaucipir and MK-6240 using the HEAD Study

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Background: Tau PET provides continuous measurements of tau tangle pathology in the human brain. However, establishing cutoffs is crucial for selecting individuals for treatment in clinical trials or practice. In the absence of postmortem data, PET cutoffs must be established using statistical methods based on what is considered normal tracer uptake. In this study, we tested the impact of various methods to determine tau positivity using two different tau PET tracers in individuals scanned head-to-head. **Methods:** We studied 147 individuals from Head-to-Head Harmonization of Tau Tracers in Alzheimer's Disease (HEAD) study with tau tangle PET scans with [^{18}F]Flortaucipir and [^{18}F]MK-6240, and amyloid-β (Aβ) PET. Tau deposition was measured with the standardized uptake value ratio (SUVR) of each agent in the Medial Temporal Lobe (MTL) and the Entorhinal Cortex (EC). To determine Tau positivity two different methods were used: >2.5 standard deviations (SD) than the mean of the young and >1.5 SD mean of the cognitively unimpaired (CU) elderly Aβ-. **Results:** When applying the cutoff >2.5 SD mean of young, [^{18}F]Flortaucipir was positive in 35 (23.8%) and 69 (46.9%) individuals in the EC and MTL, respectively. [^{18}F]MK-6240 was positive in 49 (33.3%) and 58 (39.5%) individuals in EC and in MTL. Using >1.5 SD mean of CUAβ-, [^{18}F]Flortaucipir was positive in 38 (25.9%) and 45 (30.6%) individuals in EC and MTL, while [^{18}F]MK-6240 was positive in 51 (34.7%) and 50 (34.0%) in EC and in MTL. **Conclusions:** Our findings indicate variations in tau positivity when employing different methods based on either the young or CUAβ-. Flortaucipir exhibited a higher rate of positive results when the method based on young individuals was applied in the MTL. Conversely, [^{18}F]MK-6240 showed more consistent and generally higher positivity when other methods were used for cutoff determination and/or in the EC region. Further research with a larger sample size is required to gain a better understanding of the optimal cutoff determination methods for these tracers.

Poster 26

Evaluating endothelium-dependent and -independent vasodilation in individuals with sickle cell disease

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Hemolytic anemia is a hallmark of sickle cell disease (SCD) and contributes to vasculopathy, which remains poorly understood in SCD. Microiontophoresis (MI), paired with laser speckle contrast imaging (LSCI), is a non-invasive technique for evaluating endothelium-dependent and -independent vasodilation in the cutaneous microvasculature. We used MI to characterize vascular dysfunction in SCD, demonstrating its utility as an *in vivo* biomarker of vasculopathy.

Subjects with SCD vs controls ≥18 years were recruited. MI was performed on the forearm by transcutaneous delivery acetylcholine (ACh), endothelium-dependent vasodilator, and sodium nitroprusside (SNP), NO donor that induces endothelium-independent vasodilation. Imaging was performed using LSCI. It emits light that gets backscattered by moving RBCs, causing a shift in frequency, used to calculate red cell flux (RCF). Data were analyzed using tabulation, t-test and Pearson's correlation.

We enrolled 14 controls and 13 patients with SCD. Mean age was 36 years for controls vs 29 years for patients. Females accounted for 13/14 controls vs 7/13 patients. Among those with SCD, 7 were on hydroxyurea and 3 on chronic RBC exchange transfusion.

Mean Hb was 12.6 g/dL in controls vs 10.8 g/dL in SCD. Although we expected that low RCF would be positively correlated with low Hb, we found mean baseline RCF to be similar in both groups. Mean peak RCF was similar in both groups in response to ACh but was higher in patients with SCD vs controls in response to SNP ($p=0.03$). ACh response was negatively correlated with creatinine ($r=-0.4$, $p=0.04$), while SNP response was positively correlated with HbS% ($r=0.4$, $p=0.02$).

Our findings show no difference in endothelium-dependent vasodilation, but enhanced endothelium-independent vasodilation in patients compared to controls. This is possibly due to abnormal vascular smooth muscle regulation, NO depletion, and compensatory hyperdynamic circulation caused by anemia and hemolysis in SCD. MI is a valuable tool to assess vasculopathy in SCD. Additional studies into the mechanisms are ongoing.

Poster 108

Astrocyte reactivity is associated with Alzheimer's disease biomarkers before amyloid-β deposition.

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Background: Aβ plaques are the first detectable signs of AD pathology. Our group recently demonstrated that the astrocyte activation marker, glial fibrillary acidic protein (GFAP), has a pivotal role in the association between Aβ burden and tau phosphorylation. However, the role of astrocyte activation in individuals that do not present detectable Aβ pathology is still underexplored. Here we sought to understand the association between plasma GFAP with AD biomarkers in Aβ negative individuals. **Methods:** 598 study participants [cognitively unimpaired, (CU)=200, and cognitively impaired, (CI)=398] from two cohorts (TRIAD, Canada and BICWALZ, South Korea) were assessed for Aβ-PET, plasma GFAP, p-tau217, NfL, and hippocampal volume measures. Pearson correlations between plasma GFAP and plasma p-tau217, plasma NfL along with hippocampal volume in Aβ-PET negative individuals. **Results:** Our results show that plasma GFAP levels positively correlate with plasma p-tau217 levels in Aβ-PET negative individuals in both the cohorts [TRIAD cohort: r=0.23, p=0.01; BICWALZ cohort: r=0.21, p=0.0001; **Figure: 1A, B**]. Further, the correlation between plasma GFAP with plasma NfL has also been found to be positively correlated among the two cohorts [TRIAD cohort: r=0.45, p=0.0001; BICWALZ cohort: r=0.49, p=0.0001; **Figure: 2A, B**]. We also observed that hippocampal volume, as a neurodegeneration marker, reveals a significant negative Pearson correlation with plasma GFAP levels even in Aβ-PET negative human individuals from both the cohorts [TRIAD cohort: r=-0.27, p=0.0009; BICWALZ cohort: r=-0.24, p=0.0001; **Figure: 3A, B**]. **Conclusions:** Our study of fluid biomarker-based evidence from a research-based and a memory clinic cohort provides critical insight into very early changes in astrocyte activation and its correlation with early AD biomarkers earlier than Aβ can deposit at a detectable level.

Poster 42

Geography of birth centers in the US demonstrates impacts of structural racism on childbearing health

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Birth center care, largely midwife-led, is associated with better health outcomes across all demographic and socioeconomic groups. Inequities in access persist in the US, however, particularly to the detriment of Black childbearing people who experience the worst perinatal outcomes. Studying birth center access not only calls for the understanding of their geographic distribution, shaped by variation in state-level regulation, but also a critical approach that considers the racist suppression of midwifery by public health and obstetrical fields in the 19th and 20th centuries.

Applying anti-racist frameworks, this study aims to describe the "economic geography" of birth centers in the US.

Using secondary data, we mapped birth center locations in the contiguous US (48 states and Washington, DC), socioeconomic make-up of census tracts in which they reside, and state regulatory environments. We applied multilevel logistic modeling to quantify associations between state-level regulation, tract-level demographics, and birth center presence.

Our final analytic sample included 503 birth centers. Analyses demonstrated a positive relationship between white population proportions and birth center presence; inverse associations between Black and impoverished population proportions and birth center presence; and how restrictive state-level regulations exacerbate these inequities.

Birth center locations are scarce in the US, particularly in states where higher densities of Black people reside, which also have more restrictive legislation around birth center care, illustrating modern impacts of the racialized history of obstetrics and midwife subjugation. Results demonstrated how birth center locations are biased toward white populations, and consequently how systemic racial privilege (a necessary complement to racial oppression) is being reinforced. Uprooting the racism that gatekeeps birth center and midwifery care requires not only changing state regulation, but also maximizing access in areas where systemically oppressed populations reside.

Poster 80

Good Manufacturing Practice culture of human corneal stromal stem cells for clinical applications

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Introduction: Corneal stromal stem cells (CSSCs) display wound healing potential, preventing corneal scarring in mouse models of stromal injury by reducing inflammatory cell infiltration, producing regenerative cytokines, and anti-scarring microRNAs, fostering scar inhibition and restoring native-like stromal tissue. To produce donor CSSC for clinical use, we developed Good Manufacturing Practice (GMP) compliant Standard Operation Protocol (SOP).

Methods: We used our standard corneal tissue processing protocol to obtain anterior limbal stroma, which was digested with GMP-grade collagenase to yield single cells with high viability. Using an xCELLigence system, we optimized primary cell attachment and culture medium with GMP-grade reagents, ensuring safety and proper cell growth. Cryo-storage of GMP-expanded cells was tested with GMP DMSO preservation media. Phenotypic characteristics of donor CSSC were compared under GMP versus lab-based protocols through RNA analysis of stemness markers and in vitro keratocyte differentiation assays.

Results: 22 donor corneas were used for GMP-CSSC cultures. The SOP included NB6 collagenase (Nordmark) for tissue digestion, recombinant human fibronectin (MCE) for cell attachment, GMP reagents, and CryoStor CS5 (BioLife) for cryopreservation. GMP-CSSCs showed stemness marker expression akin to lab protocols. GMP cells also differentiated to assume keratocyte features.

Conclusion: Our optimized GMP SOP produced clinical-grade human CSSCs, vital for regulatory compliance and FDA IND application.

Poster 109

Abnormal polyunsaturated fatty acid metabolism is associated with a phenotype with marked sepsis-associated acute kidney injury in pediatric sepsis

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Pediatric sepsis has overall lower mortality than adult patients, but still a sizeable proportion of children die with progressive multiorgan failure. Preclinical and observational evidence show that modulation of linoleic acid (LA) metabolism, the major omega-6 polyunsaturated fatty acid in diet, influence inflammation. LA metabolism has not yet been studied in human sepsis.

Sepsis-associated acute kidney injury (AKI) is characterized by abnormal energy metabolism, with animal models suggesting that functional recovery and survival is at least in part dependent on restoring the capacity of renal tubular epithelial cells to use fatty acid oxidation.

We hypothesize that markers of higher exposure to LA and LA-derived oxylipins will be increased in pediatric sepsis phenotype D and will be associated with organ dysfunction.
We utilized ultra-performance liquid chromatography to analyze untargeted metabolomics in a cohort of 107 children with sepsis. We categorized sepsis phenotypes as previously described into phenotypes A-D. We built a heat map featuring comparisons between pairs of phenotypes. For this study we focused on the polyunsaturated fatty acid metabolic pathways.

Phenotype D group showed higher incidence of AKI ($p < 0.05$). We found that LA was not significantly different in phenotype D compared with others. Conversely, arachidonic acid (AA) was lower in phenotype D compared with phenotypes A-C, with statistically significant difference when compared with phenotype C, along with lower AA metabolites such as 5-HETE. Metabolites of LA, both via non-enzymatic pathway (13-HODE, 9-HODE), and through CYP450 (12,13-DiHOME, 9,10-DiHOME) were significantly increased in phenotype D.

AKI is markedly increased in patients with pediatric sepsis phenotype D. While phenotype D showed no significant changes in LA levels, LA inflammatory metabolites were increased, and AA was decreased along with its metabolites. In summary, children with sepsis with phenotype D, showed metabolism abnormalities with increase of LA metabolites independent of AA.

Poster 81

Curtailing Ferroptosis: The Inhibitory Role of Ferroloxins in Phosphatidylethanolamine Oxidation via the 15LOX/PEBP1 Complex, both In Vitro and In Vivo

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Ferroptotic cell death program triggered by imbalanced metabolic coordination of iron, glutathione and lipid peroxidation has been implicated in the pathogenesis of many diseases including neurodegeneration, heart and kidney diseases, and cancer. Manipulation of ferroptosis presents a promising attractive strategy for therapy and ferroptosis has become a potential intervention target in these diseases. We demonstrated that complex of 15-lipoxygenase (15LOX) with phosphatidylethanolamine (PE)-binding protein 1 (PEBP1) steers the function of 15LOX towards ferroptosis-specific generation of oxygenated PE species and induction of ferroptosis. We developed a new anti-ferroptotic agents, Ferroloxins. In this study we demonstrated that Ferroloxins inhibit 15LOX/PEBP1 catalyzed oxidation of PE and prevent/mitigate ferroptosis.

Poster 110

Post translational glycosylation of apoeε3 is associated with brain amyloid-β burden and neurodegeneration in Alzheimers disease

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Apolipoprotein E (ApoE) is a glycoprotein with central roles in lipid metabolism and neurodegeneration. However, the post-translational addition of a glycan sugar to the original structure of ApoE is associated with ApoE functional changes. Previous studies have shown that high levels of ApoE glycosylation are associated with deficits in the clearance of amyloid-β (Aβ) from the brain. Because some ApoEε3 carriership individuals develop Alzheimer's disease (AD) pathology and others do not, in this study we aimed to investigate the hypothesis that the levels of ApoEε3 glycosylation can distinguish between ApoEε3 carriers with and without amyloidosis. We assessed 188 individuals from the Alzheimer's Disease Neuroimaging Initiative [ADNI; 91 CU, 97 MCI] with ApoEε3 measures in the cerebrospinal fluid (CSF). We divided individuals according to their APOE glycosylation levels based on terciles. We tested the association between biomarkers using linear regression models accounting for age and sex, and ANCOVA to compare groups. ApoEε3 glycosylation levels were negatively associated with levels of amyloid-β 42 in the CSF (β = -0.218, p = 0.013). Voxel-wise linear regression analysis showed a positive association of ApoEε3 glycosylation levels with brain amyloid burden, especially at the neocortical area (β = 0.344, p = 0.001). We also observed that the association of ApoEε3 glycosylation with brain Aβ burden, was present in cognitively unimpaired (β = 0.397, p = 0.014) and mild cognitive impaired (β = 0.350, p = 0.016) individuals. Moreover, ApoEε3 glycosylation levels were negatively associated with an annual reduction in brain volume only in individuals classified as Aβ positive (β = -0.311, p = 0.023). No associations of ApoEε3 glycosylation with the levels of total tau and phosphorylated tau 181 were observed. Taken together, our results demonstrate that high glycosylation levels of ApoEε3 are associated with increased deposition of Aβ in the brain and decreased brain volume over time, particularly in Aβ positive individuals.

Poster 43

Supporting Home Visitors Infant Mental Health Knowledge Through Professional Collaboration

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This study investigates ways Early Head Start (EHS) Home Visitors (HVs) participation in a Professional Learning Community (PLC) may contribute to growth in their Infant Mental Health (IMH) knowledge and self-efficacy to implement these competencies into practice. PLCs have been recognized as a professional development design that supports participants' ability to work collaboratively (Yin et al., 2019), better use instructional practices (Tam, 2015), and enhance learner success (Vescio et al., 2008). Partnering with Peers (PWP) was created to support HVs developing IMH knowledge through guided lectures, community sharing, and real-world applications.

PWP aims to cultivate space for reflection where HVs can express themselves, their work, and their lived experiences while being candid about the challenges they may face personally and in the work. We follow the HVs wondering, creating a space for personal growth and collaboration. HVs are encouraged to interrogate the complexity of the local sociopolitical climate and the pervasive effects racism has on the families we serve. We hope to generate personal and collective strategies to cultivate racial equity in our everyday work.

Surveys were used to collect information twice (pre/post) on HVs (N=19) self-efficacy to implement IMH competencies and their emotional regulation (ER). Findings suggest HVs who participated in PWP identified that they felt generally confident in their ability to use IMH practices before beginning PWP, which increased to highly confident at completion. HVs IMH self-efficacy was significantly related to their ER ($\beta=0.94$, $p<0.00$). Further, HVs self-efficacy to implement IMH competencies ($p<0.00$) and their ER significantly increased ($p<0.01$) after PWP.

These are interesting and important findings when considering how ER relates to relationship development with children and families and the successful application of IMH competencies. The current study offers insights into how a PLC can be used to support the development of HVs IMH knowledge and confidence to implement this knowledge into their work.

Poster 27

Ocular bioavailability of methotrexate as a potential drug for retinitis pigmentosa treatment

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Methotrexate (MTX) is a methylated folic acid analogue frequently applied in chemotherapy and the treatment of autoimmune disorders. In our recent work published in 2020, we reintroduced MTX as a potential therapeutic candidate for RHO-associated autosomal dominant retinitis pigmentosa (adRP) which selectively clears misfolded P23H rhodopsin mutant protein via lysosomal degradation. Excitingly, intravitreal injection of MTX showed retinal protective effects in the $Rho^{P23H/+}$ knock-in mice and a phase I clinical trial in adRP patients carrying RHO mutations. However, as frequent intravitreal injections can be challenging for the development of a preventive treatment of RP, the need to understand MTX retinal bioavailability via systemic administration arises.

In this work, we aim to evaluate the ocular accumulation and clearance of MTX following both bolus and routine administration to murine models. Following intravenous tail injections to both WT and 3 to 4-month-old $Rho^{P23H/+}$ mice, tissue collection and homogenization, the dose-dependent (1, 3, 5, 10 mg/kg) MTX concentration were evaluated in isolated retina, eye, liver, and plasma by analytical high performance liquid chromatography (HPLC). We observed a dose-dependent ocular MTX concentration change that peaks at 3 mg/kg in both murine models, with the $Rho^{P23H/+}$ mice presenting significantly lower ocular bioavailability when compared with age-matched WT mice. Following two-week daily dosage of MTX, WT and $Rho^{P23H/+}$ mice were analyzed by ocular coherence tomography, electroretinogram, and histology for the evaluation of ocular safety and efficacy in preserving retinal morphology and function. The preliminary results indicate that the toxicity of MTX is not significant in adult mice, while it strongly affects the CNS development in PND15-30 young individuals. To recreate vision preservation by non-invasive administration of MTX, lower drug dose and intervals between injections are desired.

Poster 28

Sex-based differences in proximal tubule endocytic capacity in mice

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Women are more protected from acute kidney injury, and chronic kidney disease (CKD) in women progresses more slowly to end-stage renal disease compared to men. CKD involves changes in the integrity of the glomerular filtration barrier with consequent proteinuria, the loss of protein in the urine. The kidney proximal tubule (PT) efficiently reclaims normally filtered levels of albumin and proteins via receptor-mediated endocytosis facilitated by megalin and cubilin. Recent transcriptomic studies in mice have shown differences in the expression of megalin and cubilin between males and females and across the S1, S2, and S3 sub-segments that comprise the PT. To determine if there are sex-based differences in the capacity to recover filtered proteins by the PT, urine and kidney tissue were collected from male and female 13-week-old C57BL/6 mice. Female mice had lower levels of urinary albumin excretion when normalized to creatinine than males. Female mice had greater expression of total megalin and cubilin by Western Blot. We used imaging-based approaches to quantify the expression and distribution of albumin, megalin, and cubilin in SGLT2-positive S1 and SGLT2-negative, OAT1-positive S2 segments. S1 had greater intensity of subapical albumin staining in both males and females. However, females had greater intensity of albumin staining in S2 normalized to S1 compared to males. Male and female mice had similar levels of megalin expression and surface localization in S1, but female mice had greater expression and surface localization of megalin in S2. Males had greater colocalization of megalin with lysosomal marker, LAMP1. In both males and females, the colocalization of megalin with LAMP1 decreased from S1 to S2. Together, these data suggest that, in female mice, S2 has greater endocytic capacity and contributes more to the uptake of normally filtered albumin than in males. Current studies are focused on incorporating these sex-based differences into a multiscale mathematical model of protein uptake along the length of the PT.

Poster 82

Differential tumor immune microenvironment coupled with tumor progression or tumor eradication in HPV-antigen expressing squamous cell carcinoma (SCC) models.

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Human papillomavirus (HPV) is an etiological factor of head and neck squamous cell carcinoma (HNSCC). To check the effect of differential immune tumor microenvironment (TME), we established mouse models (C-225 and C-100) transplantable into C57BL/6 recipients by expressing HPV16 E6 and E7 in an SCC tumor cell line. We found that C-225 elicited complete eradication in C57BL/6 mice, whereas C-100 grew progressively. We examined the immune TME using flow cytometry and found that eradicated or growing tumors exhibited differential immune profiles. Surprisingly, the percentage of CD8 and CD4 tumor-infiltrating lymphocytes (TILs) was much higher in growing (C-100) than eradicated (C-225) tumors. However, the TILs upregulated PD-1 and LAG-3 more potently and exhibited impaired effector functions in growing tumors compared to their counterparts in eradicated tumors. C-225 TME is highly enriched with myeloid cells, especially polymorphonuclear (PMN) myeloid-derived suppressor cells (MDSC), whereas the percentage of M-MDSC and tumor-associated macrophages (TAMs) was much higher in C-100 TME. The complete eradication of C-225 depended on CD8 T cells and elicited anti-tumor memory responses upon secondary tumor challenge. We employed DNA sequencing to identify differences in the T cell receptor of peripheral blood lymphocytes pre- and post-secondary tumor challenge. Lastly, C-225 and C-100 tumor lines harbored different somatic mutations. Overall, we uncovered differential immune TME that may underlie the divergent outcomes of anti-tumor immunity. Our experimental models may benefit in pinpointing tumor-intrinsic versus host-intrinsic differences in orchestrating an immunosuppressive TME in HNSCCs and for identifying targets that render tumor cells vulnerable to immune attack.

Poster 83

Role of G0/G1 Switch Gene 2 in Transforming Growth Factor β1-Induced Hypertrophy of Ligamentum Flavum

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Lumbar spinal stenosis (LSS) is a major spinal disorder in elderly patients contributing to back pain. Hypertrophy of ligamentum flavum (HLF) is the main etiology of LSS. Studies have shown several histological changes in thickened LF including a loss of elastic fibers and increased collagen fibers and proteoglycans, led by upregulation of collagenases, cell differentiation to myofibroblasts in addition to several growth factors such as transforming growth factor-beta (TGF-β), inflammatory cytokines, angiogenesis factors etc. contributing to pathological changes in HLF. G0/G1 switch gene 2 (G0S2), first identified in activated lymphocytes to regulate lipid metabolism to control cell proliferation, is implicated in the regulation of diverse biological and pathological processes such as cell proliferation, apoptosis, inflammation, metabolism, and carcinogenesis. Recent studies have shown that G0S2 mediates the renal inflammation and fibrosis leading to chronic kidney disease, however its role in hypertrophy of ligamentum flavum is not known. Here, we identified that G0S2 expression is increased upon TGF-β1 stimulation of LF cells and regulates the expression of genes involved in fibrogenesis. Stimulation of control or hypertrophic LF cells with TGF-β1 (5 ng/ml) induced the expression of *G0S2* showing that G0S2 is implicated in fibrotic signaling in LF cells. TGF-β1 increased the expression of *TGFB1*, collagen-1 (*COL1*), versican (*VCAN*), alpha smooth muscle actin (α-SMA/*ACTA2*) in both normal and HLF cells revealing the induction of extracellular matrix fibrosis and myofibroblast differentiation by TGF-β1. Knockdown of G0S2 by using siRNA significantly inhibited the TGF-β1 mediated expression of *TGFB*, *VCAN* and *ACTA2* suggesting G0S2 as a potential regulator of pathologic development of hypertrophy of LF. Future studies are necessary to establish the molecular mechanisms behind G0S2 regulation of fibrogenesis and its regulation of HLF.

Poster 126

Accessible Autonomous Transportation and Services: Design considerations from the perspective of consumers and providers

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To understand the priorities and preferences of people with disabilities (PwDs) and older adults regarding accessible Autonomous Vehicles (AVs) to address existing transportation barriers. The research establishes a foundation for a more equitable and accessible transportation landscape through AV technology integration. Two national surveys, "Voice of the Consumer (VoC)" and "Voice of the Provider (VoP)," were conducted to gather feedback from accessible AV consumers and providers respectively in the United States (US). This US-based study focused on PwDs and older adults who may face transportation challenges and those who provide or design AV solutions. The 922 consumers and 45 providers in the surveys encompassed a diverse range of disability types, caregiver roles, and age groups. The main outcomes are consumer usage needs and provider preferences for features inaccessible to autonomous transportation. Patterns in usage needs and feature preferences through a two-step clustering algorithm were applied, after the descriptive analysis of participant demographics and their responses. Participants strongly preferred AV features enhancing personal transportation, especially for rural medical appointments. Most sought comprehensive AV automated features. Wheelchair users emphasized accessible entrances, particularly for lower-income brackets ($25,000 - $49,000). Provider priorities are closely aligned with consumer preferences, reinforcing content validity. The study highlights the importance of prioritizing wheelchair accessibility in AVs and improving access to medical appointments, especially in rural and low-income communities. Implications include developing inclusive AV services for PwDs and underserved populations.

Poster 44

Mental health among sexual and gender minority and cisgender heterosexual autistic young adults

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One in 45 US adults is autistic, ~35% of whom identify as a sexual-and-gender minority (SGM). Cisgender-heterosexual (CisHet) autistic people experience stigma and negative mental health effects; however, less is known about mental health outcomes among people who are autistic and SGM, particularly among young adults. As such, this study aimed to examine patterns of mental health and service utilization among CisHet and SGM autistic young adults.

Autistic participants (18-25 years) completed self-report measures of sociodemographics, mental health history, and current anxiety and depression symptoms. For the current study, participants were grouped into CisHet and SGM groups.

Of 210 autistic young adults (*M*=22.5 years), 91 participants comprised the SGM group, and 119 adults comprised the CisHet group. There were no group differences in race/ethnicity (78% White Non-Hispanic/Latine). The SGM group was more likely to have higher education, be in a committed relationship, and prefer 'identity-first' language, whereas the CisHet group had less education, were single/dating, and preferred 'person-first' language. Overall, SGM autistic adults endorsed more mental health diagnoses (*M*=3.1) than CisHet autistic adults (*M*=2.3). Specifically, both groups reported high rates of bipolar disorder, OCD, and ADHD; however, the SGM group was more likely to report higher rates of depression (81% vs 44%), anxiety (88% vs 59%), and PTSD (39% vs 20%). Both groups were equally likely to be utilizing psychiatric interventions.

Nearly half (43%) of young autistic adults identified as an SGM. Both groups reported significant mental health challenges and comparable service utilization; however, relatively fewer SGM participants were using psychiatric services despite having more mental health challenges. Ultimately, it is critical that future work identify barriers and ways to support the mental health needs of all young autistic adults, and with an intersectional approach toward SGM autistic people.

Poster 29

A Novel Vascular Scaffold for Focal Delivery of Antiplatelet Therapy

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More than 1.8 million percutaneous coronary interventions (PCI) are performed annually to treat coronary artery stenosis. Stent thrombosis (ST) is a catastrophic mechanism for stent failure, comprising 11% of cases of acute myocardial infarction, and carries high morbidity and mortality; its prevention requires the use of prolonged dual antiplatelet therapy (DAPT), which significantly increases bleeding risk, rendering PCI therapy of prohibitively high risk in certain patients. A contemporary stent system that provides local antiplatelet activity, obviating the need for systemic DAPT to mitigate bleeding risk, does not exist.

To fill this urgent unmet need, we have developed such a stent: the ticagrelor-coated stent (TCS). The TCS is coated with the potent antiplatelet agent ticagrelor, utilizing self-assembled monolayer technology, for which we have established in vitro proof of concept. In a porcine ex vivo AV fistula model, TCS was placed in alternating series adjacent to uncoated bare metal stents (BMS). No thrombus was seen on the TCS compared to the BMS. Platelet adherence and micro-thrombi presence were significantly reduced (17.8% TCS area vs 55% BMS area; $p<0.0005$). Notably, inflammation, measured by neutrophil and monocyte adherence, was also reduced by approximately 10-fold on the TCS vs. the BMS [neutrophil 127 cells/mm2 vs. 941 cells /mm2 ($p<0.005$); monocyte 21 cells/mm2 vs. 210 cells/mm2 ($p<0.001$)]. We also implanted the TCS in rabbits for 35 days and demonstrated superior performance compared to BMS.

Most recently, we implanted the TCS in a pig coronary artery and found it widely patent and thrombus-free without systemic DAPT. Thus, the TCS provides local antiplatelet activity to prevent ST and minimize bleeding risk by eliminating the need for systemic DAPT. Moreover, this stent is expected to provide a safe revascularization option for patients with limited access to or who cannot take daily medications. Long-term TCS efficacy studies in the porcine model are currently underway.

Poster 30

Daptomycin tolerance in vancomycin-resistant *Enterococcus faecium* isolate from patients with recurrent bloodstream infections

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Enterococcus faecium is a gastrointestinal tract commensal that has become a concerning nosocomial pathogen. *E. faecium* is resistant to many first-line antibiotics and most clinical isolates are also vancomycin-resistant (VREfm). As such, VREfm bloodstream infections (BSIs) are challenging to treat, and a proportion of patients develop recurrent disease despite appropriate therapy. How VREfm adapts within its host to evade eradication and cause recurrent infection remains an important clinical question. Daptomycin is the recommended VRE antibiotic in our hospital system, and although resistance in the community is relatively uncommon, daptomycin tolerance has been documented in clinical enterococcal isolates. We hypothesize that the development of daptomycin tolerance within the human host may drive recurrent VREfm BSIs. Through prospective banking of all VREfm-positive blood cultures across 3 hospitals, we collected 53 serial VREfm isolates from 14 patients with recurrent BSIs. Recurrent infections constituted ~10% of the sampled VREfm BSIs, and 8/14 patients experienced multiple recurrences. In 13/14 patients, all collected isolates differed by <20 single nucleotide polymorphisms, which suggests that within an individual, recurrent infections are caused by a single bacterial strain rather than reinfection with a novel lineage. Additionally, <25% of the isolates were daptomycin resistant, and despite a high probability of exposure, daptomycin resistance did not uniformly develop over the course of repeated BSIs. Time-kill assays on a subset of recurrent VREfm BSI isolates showed that daptomycin tolerance increased in isolates collected from later BSIs as compared to the initial infection, and we are working to assess this phenomenon across the entire cohort. These results, in combination with additional planned comparative genomic analyses, will provide a large, systematic study of daptomycin tolerance and the associated genetic adaptations that emerge within a human host to facilitate persistent VREfm colonization and recurrent infection.

Poster 127

CRISPRa Based Upregulation of Runx2 or Sox9 Expression in Goat BMSC for Regeneration of the TMJ

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The temporomandibular joint (TMJ) is susceptible to osteoarthritis (OA) (TMJ-OA) with a third of patients undergoing total TMJ replacement having TMJ-OA without comorbid conditions. Currently, there are no FDA approved allograft alternatives to total joint replacement. Recently clustered regularly interspaced short palindromic repeats (CRISPR) epigenome editing has been utilized to engineer bone marrow stem cells (BMSCs) with enhanced therapeutic properties. In this study, we examine the potential of utilizing CRISPR upregulation (CRISPRa) of Runx2 or Sox9 expression in goat BMSCs to enhance their therapeutic potential of regeneration of the TMJ.

In this study, we first created CRISPR epigenome editing lentiviral that express 1) dCas9-VPR-PuroR or 2) gRNAs that target the promotor region of Runx2 or Sox9, or a non-target gRNA sequence that does not match the goat genome (NT). Next, we performed a kill curve experiment to determine the puromycin dosage for selection of VPR expressing BMSCs (n=5 donors). Finally, BMSCs were co-transduced with VPR and gRNA lentiviral vectors and gene expression of Sox9 or Runx2 was measured via RT-qPCR 7 days after transduction.

Growth media supplemented with 1 μg/mL was demonstrated to be the minimum dose of puromycin required to kill BMSCs after 3 days, and was thus used in all succeeding steps. BMSCs transduced with gRNAs targeting the Runx2 promotor region exhibited significantly higher expression ($p<0.05$) of Runx2 when compared to nontarget (NT) controls (8.68 ± 0.17 fold). Similarly, BMSCs transduced with gRNAs targeting the Sox9 promotor region exhibited significantly higher expression ($p<0.05$) of Sox9, when compared to NT controls (10.27 ± 0.34 fold). Together, these results demonstrate that CRISPR epigenome editing of BMSC can be utilized to upregulate expression of Runx2 and Sox9, key regulators of osteogenic and chondrogenic differentiation, respectively and suggests this approach may be utilized in therapeutic strategies for TMJ regeneration.

Poster 84

Changes in Rifampin Resistance-Determining Region (RRDR) of RpoB Cause Mutation-Specific Phenotypic Changes in *Enterococcus faecium*

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Vancomycin-resistant *Enterococcus faecium* (VREfm) is a primary cause of bloodstream infections (BSI) in immunocompromised patients. As enterococci are well adapted to the GI tract, to successfully colonize the bloodstream, VREfm-BSIs must evolve separate traits. Investigating these bloodstream-adapted changes can help us understand how VREfm is able to cause such persistent and deadly infections.

Preliminary studies of VREfm-BSI isolates in UPMC revealed mutations in *rpoB* as promising candidates for investigation. Mutations in *rpoB*, usually correlated with rifampicin resistance, also impact cephalosporin resistance in enterococci. However, the effect of mutations in *rpoB* on general enterococci gene expression remains to be explored.

To explore the phenotypic consequences of mutations in *rpoB,* we isolated four isogenic mutant strains with changes in the rifampin resistance-determining region (RRDR) of *rpoB*. These mutations were Q473K, G482V, H486Y, and S491L. We performed phenotypic testing on the wild type parent strain and the four *rpoB* mutants to measure various phenotypic changes.

In testing the growth rate, we found that the isolates with G482V and H486Y mutation had a faster doubling time compared to the wild type. The other two mutations led to increased fitness cost for *E. faecium.* Next, we tested the isolates antibiotic susceptibility against six antibiotics: rifampicin (RIF), vancomycin (VAN), daptomycin (DAP), meropenem (MEM), ceftriaxone (CRO), and ampicillin (AMP). All mutant isolates had higher RIF MICs but lower AMP MICs compared to the wild type. In addition, we found distinct *rpoB* allele-specific effects on DAP MIC. Interestingly, we found that the isolate with S491L mutation in had significantly higher tolerance to isopropanol compared to the wild type or other isogenic mutants.

Overall, we found that despite occurring in the RRDR region of *rpoB*, the different mutations we studied resulted in different phenotypic effects in an allele-specific manner, highlighting the diversity of possible phenotypic consequences of mutations in *rpoB*.

Poster 128

The effect of polyploidy and mating system on floral size and the pollination niche in Brassicaceae

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Polyploidy, a major evolutionary process in flowering plants, is expected to impact floral traits which can have cascading effects on pollination interactions, but this may depend on selfing propensity. In a novel use of herbarium specimens, we assessed the effects of polyploidy and mating system on floral traits and the pollination niche of 40 Brassicaceae species. We combined data on mating system (self-compatible or self-incompatible) with inferred ploidy level (polyploid or diploid) and use phylogenetically controlled analyses to investigate their influence on floral traits (size and shape) and the degree of pollination generalism based on the frequency and the richness of heterospecific pollen morphospecies captured by stigmas. Flower size (but not shape) depended on the interaction between ploidy and mating system. Self-incompatible polyploid species had larger flowers than self-incompatible diploids but there was no difference for self-compatible species. The breadth of pollination niche (degree of generalism) was not affected by ploidy but rather strongly by mating system only. Self-incompatible species had more stigmas with heterospecific pollen and higher heterospecific pollen morphospecies richness per stigma than self-compatible species, regardless of their ploidy. Our results demonstrate that mating system moderated the influence of ploidy on morphological features associated with pollination generalism but that response in terms of heterospecific pollen captured as a proxy of pollination generalism was more variable.

Poster 111

Synaptic gene variants are associated with post-traumatic epilepsy and long-term outcome after severe traumatic brain injury

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Post-traumatic epilepsy (PTE) occurs in up to 1/3 of severe traumatic brain injury patients (sTBI). PTE is independently associated with impoverished long-term outcomes. Alterations in synaptic neurotransmission have been linked to the development of PTE, however underlying genetic etiologies remain unknown. We hypothesize that single nucleotide polymorphisms (SNPs) of synaptic genes are associated with PTE and/or outcomes in sTBI patients. Subjects were prospectively enrolled through University of Pittsburgh BTRC between 2002-2013, with corresponding PTE status extracted from electronic medical record. DNA was extracted from 385 patients. SNPs from *AP2M1, CLTA, CLTC,* and *SYT1* were genotyped using the Human Core Exome. Outcome up to 24mo post-injury was measured using Glasgow Outcome Scale (GOS) and Disability Rating Scale (DRS). PTE occurred in 28% (n=57) of patients. 38 SNPs across 4 genes were identified. Multivariate logistic regression, controlling for sex, age, and GCS found *AP2M1* rs8478 and rs2231224 minor allele variants significantly increased the odds of PTE ($p<0.05$, OR=2.21 and 2.19, respectively). *CLTA* rs4879960 major allele variant significantly reduced odds of poor outcome on 6-24mo DRS ($p<0.05$, $OR<0.6$). 6 *Syt1* minor allele variants significantly reduced odds of poor outcome on 12mo GOS (rs1405499, rs1918193, rs1918191, rs1245804, rs1245824 and rs2272500, $p<0.05$, $OR<0.5$). This study found an association between three synaptic genes and PTE or long-term outcome in a sTBI population. Identification of these genetic variants may improve early identification of patients at high-risk for PTE. Further work aims to determine the implication of these polymorphisms on pathological mechanisms.

Poster 85

ATR promotes mTORC1 activation via de novo cholesterol synthesis in p16-low cancer cells

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DNA damage and cellular metabolism are intricately linked with bidirectional feedback. Two of the main effectors of the DNA damage response and control of cellular metabolism are ATR and mTORC1, respectively. Prior work has placed ATR upstream of mTORC1 during replication stress, yet the direct mechanism for how mTORC1 is activated in this context remain unclear. We previously published that p16-low cells have mTORC1 hyperactivation, which in part promotes their proliferation. Using this model, we found that ATR, but not ATM, is upstream of mTORC1 activation via de novo cholesterol synthesis and is associated with increased lanosterol synthase (LSS). Indeed, p16-low cells showed increased cholesterol abundance. Additionally, knockdown of either ATR or LSS decreased mTORC1 activity. Decreased mTORC1 activity due to ATR knockdown was rescued by cholesterol supplementation. Finally, using both LSS inhibitors and multiple FDA-approved de novo cholesterol synthesis inhibitors, we found that the de novo cholesterol biosynthesis pathway is a metabolic vulnerability of p16-low cells. Together, our data provide new evidence coupling the DNA damage response and cholesterol metabolism and demonstrate the feasibility of using FDA-approved cholesterol-lowering drugs in tumors with loss of p16.

Poster 31

Development and Characterization of a Syngeneic Mouse Cell Line Representing Invasive Lobular Breast Carcinoma

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Invasive lobular carcinoma (ILC) accounts for 10-15% of all breast cancers, making it the second most common histological subtype after breast cancer of no special type (NST), also called invasive ductal carcinoma (IDC). Initially, ILC is associated with a good prognosis and shows a better response to adjuvant hormonal therapy compared to IDC. However, patients with ILC often experience a worse long-term outcome. Over 90% of ILCs are estrogen receptor positive (ER+) and do not express functional E-cadherin (CDH1). Despite these characteristics, ILC is understudied due to the limited availability of ILC models. Therefore, this study aims to create a syngeneic mouse cell line model that mimics ILC.

We created an ER+ ILC-like model using the SSM3 cell line, derived from an ER+ mouse mammary tumor in STAT1-deficient mice. To mimic a hallmark feature of ILC, we used CRISPR-Cas9 to knock out *Cdh1*. We conducted *in vitro* assays to compare the effects of *Cdh1* knockout and 3D culture with 2D monolayers in terms of cell proliferation, migration and sensitivity to estrogen. We generated bulk RNA-seq data to systematically explore transcriptomic changes and pathway enrichments in the *Cdh1* KO model. In an *in vivo* study, we injected the *Cdh1* KO ILC-like cell line and the SSM3 wildtype (WT) cell line into mice to compare tumor growth.

In vitro experiments with the *Cdh1* KO model revealed increased 2D growth of SSM3 cells but promoted anoikis resistance and altered cell morphology in a 3D environment. Analysis of the RNA-seq data identified distinctive gene expression profile changes in the *Cdh1* KO model in a 3D culture environment compared to the 2D culture, enriching for hypoxia, cholesterol homeostasis, and KRAS signaling pathways, which are important features of the tumor microenvironment contributing to tumor progression. Initial results from the *in vivo* study showed faster tumor growth in mice injected with the SSM3 *Cdh1* KO cell line.

The ER+ ILC-like mouse cell line model has the potential to be a crucial tool in studying ILC. Its development could lead to more effective endocrine and immunotherapies for ILC.

Poster 129

Cross-linked sodium alginate: An ecofriendly versatile solution for marine pollution

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The 20th and 21st centuries are often called the "Plastic age" owing to the omnipresence of these materials. The chemical characteristics of plastic have made it very practical and sturdy while also making it challenging to discard. It is estimated that nearly 33 billion pounds of plastic enter the marine environment every year. Plastic pollution is a significant problem that harms the marine ecosystem as it endangers the health of marine species; almost 1,000 species of marine animals are already impacted by ocean pollution. In addition to reduced use and recycling of plastics, development of marine degradable bioplastic is a suggested solution to limit the pollution. In the current work, we focus on development of cross-linked sodium alginate films - a hydrophilic polysaccharide derived from algae as a replacement for synthetic plastics that can triggerably degrade on contact with salt water into benign particles. The trigger able degradation mechanism relies on the replacement of divalent cations that serve as electrostatic cross-linking sites with monovalent ions (e.g., Na+) in salt water. We have successfully developed sodium alginate films by solution casting technique using water as solvent. The current focus is on improving the water vapor permeability of the developed film, so that they can be used as single-use plastic packaging.

Poster 112

Acceptability of a brain-injury tailored yoga and meditation program amongst female concussion patients

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Although concussion symptoms resolve within 4-6 weeks, for a "miserable minority" of patients, persistent post-concussion symptoms (PPCS) such as headaches, fatigue, & mood disturbances, persist for months, if not years. Rehabilitation of this group-who are often female-is crucial for reducing health and gender disparities in concussion recovery. A recent report by the National Center for Complementary and Integrative Health, showed that the use of complementary health approaches e.g., yoga & meditation, has increased substantially in the last two decades. Little is known, however, about the acceptability of these health approaches for female concussion patients, who are most vulnerable to PPCS. In this study, we evaluate the acceptability of a brain-injury tailored yoga and meditation program for female concussion patients.

An online survey of women in PINK Concussions, a peer-ran social media support network for female concussion patients, was conducted. Along with demographic and concussion-related questions, participants were asked about their interest and reasons for participating in the tailored program. Responses were reviewed and categorized into themes.

There were 434 respondents to the survey, of which 117 (26.96%) completed all survey questions. A majority (n=97;82.9%) were interested in the tailored program, for reasons including prior yoga experience (n=30), health/wellbeing benefits (n=27), and balance/healing/mindfulness (n=23). For disinterested participants (n=14;11.9%), reasons included: physical disabilities (n=6), time constraints (n=3), and enrollment in similar programs (n=5).

The majority of the PINK Concussion participants were interested in a tailored yoga and meditation program; indicating high acceptability of the complementary health approach for this group of patients.

Poster 86

Characterizing donor susceptibility to Epstein-Barr Virus infection in the nasopharynx using organotypic rafts

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Epstein-Barr virus (EBV) is a ubiquitous gammaherpesvirus that chronically infects most humans. Approximately 95% of adults become seropositive for EBV by age 18, however, despite its prevalence, a subset of individuals are at elevated risk of developing an EBV-associated cancer. In specific regions of the world (e.g. Southeast Asia), epithelial pathologies like EBV-associated nasopharyngeal carcinoma (NPC) are endemic and far more common. EBV-associated NPC is characterized by a latent and clonal EBV infection, where oral IgA antibodies to EBV lytic proteins are observed to increase several years prior to NPC diagnosis. Thus, increased lytic burden from EBV at the nasopharyngeal mucosa is considered a risk factor. Conventional 2-D cell culture cannot replicate the tissue microenvironment that exists in the nasopharynx and does not recapitulate EBV's differentiation-dependent lytic infection program observed in stratified epithelium like organotypic rafts from oral keratinocytes. Here, we have established 3-D organotypic rafts using conditionally reprogrammed cells (CRCs) from patients to model EBV *de novo* infection in the nasopharyngeal epithelium. Primary nasopharyngeal cells were collected from consenting adults undergoing skull-base surgery without nasopharyngeal co-pathology. We have established a nasopharyngeal CRC cryobank from 29 total donors to further characterize the EBV life cycle within the nasopharynx. Here we demonstrate that nasopharyngeal organotypic rafts can support EBV *de novo* infection, that multiple donor cryopreserved CRCs can be thawed and differentiated, and that nasopharyngeal rafts can be used to identify host genes encoding EBV restriction factors. We have developed a molecular diagnostic panel to identify cells undergoing lytic or latent EBV replication. Using permissive cell line as benchmarks, we have optimized single cell RNA-sequencing (scRNA-seq) analyses to profile the spectrum of EBV genes expressed among different cell types in the natural host. We are investigating variation in susceptibility to *de novo* EBV infection among different cell types.

Poster 87

The cancer-risk variant of EBNA1 improves nasopharyngeal carcinoma risk prediction in an EBNA1 IgA assay

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Antibodies to Epstein-Barr virus (EBV) proteins (EBNA1, VCA, BNLF2b) can diagnose early-stage EBV-associated nasopharyngeal carcinoma (NPC). We have shown that IgA against EBV nuclear antigen 1 (EBNA1) alone can predict incident cases in NPC high- and intermediate-risk populations up to 4 years before diagnosis. In the present study, we evaluated several mammalian-expressed EBNA1 antigens to determine the sequence requirements for IgA detection by our NPC risk prediction assay. NPC tumor-associated (V-val) and prototypic (P-ala) EBNA1 sequences which differ by at least 15 amino acids in the N- and C-terminus were resolved in a denaturing immunoblot assay to survey IgA and IgG in sera collected from individuals who later developed NPC and matched healthy controls from two independent prospective cohorts from NPC high-risk (Singapore) and intermediate-risk (Shanghai, China) populations. At 95% sensitivity, IgA against EBNA1 V-val yielded a 94.9% specificity compared to 86.1% for P-ala. EBNA1 deleted for the glycine-alanine repeat (dGAr) reduced IgA test-positives in healthy controls by up to 22.7% and optimized cut-offs better distinguished incident cases from controls. Incident NPC sera reacted more strongly to the C-terminus compared to healthy controls, but the C-terminal domain alone (a.a. 390-641) was not sufficient in reproducing the specificity of the EBNA1 dGAr construct (84.8% vs. 92.4%, respectively, at 95% sensitivity). Although EBNA1 IgA was present in healthy sera, most epitopes localized to the immunodominant GAr domain. Incident NPC cases showed reactivity that extended to epitopes in the N- and C-termini. We conclude that a refined EBNA1 antigen deleted for the GAr with unique residues representing EBV isolates from NPC tumors are key criteria for optimized specificity in our NPC risk prediction assay which measures linear epitopes and identifies high-risk individuals up to a 4-year sojourn time.

Poster 45

Subjective Memory and Perceived Risk of Alzheimer's Disease Among Older Black Adults

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Objectives: Given the disparities in access to diagnoses and treatment in cognitive decline, there is more reason to take note of subjective memory complaints among African American adults. One way to better understand its relevance is through further exploration into how important memory complaints are to one's perceived risk of Alzheimer's disease. Therefore, the objective of the current study was to investigate the associations among subjective memory complaints and perceived risk of Alzheimer's disease among older African American adults.

Methods: This secondary data analysis included 341 African American adults aged 45 and older. Subjective memory complaints were measured with the Frequency of Forgetting Scale. Multiple linear regression models were used to examine the relationship between perceived Alzheimer's disease risk and total score of subjective memory assessment after controlling for demographics. The interaction between subjective memory assessment with sex and education were also investigated.

Results: Mean age of participants was 60, male participants reported better subjective memory performance than female participants, and females reported a slightly higher chance of developing Alzheimer's disease in the next 10 years than male participants. Regression analyses showed that reporting female sex moderated the association between perceived Alzheimer's disease risk and subjective memory complaints.

Conclusion: Results indicate that perceptions of Alzheimer's disease risk were strongly associated with sex and subjective memory complaints or potential presence of cognitive symptoms. Future research should be expanded to include race-based cognitive ability, as well as gender-based cognitive ability.

Poster 32

Unveiling TGFB3 Upregulation in Systemic Sclerosis: Insights from Chromatin Accessibility and Transcription Factor Analysis

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Systemic sclerosis is a heterogeneous autoimmune disease with an unknown etiology. A marked increase in TGF-beta3 immunoreactivity is detected within the scleroderma skin compared to healthy individuals.

In our study, scRNA-Seq and scATAC-Seq analyses have provided additional insights into the role of TGFB3 in systemic sclerosis. High expression of TGFB3 is observed selectively in scleroderma skin and lung myofibroblasts. Furthermore, within the TGFB3 gene, an open chromatin region (OCR1) shows increased accessibility in myofibroblasts.

ChromBPNet is an analysis tool that can effectively decode the multi-resolution structure of chromatin profiles produced by the cooperative binding of transcription factors. Using ChromBPNet to analyze ATAC-seq data from IMR90 human lung fibroblasts treated with TGF-beta and control, we identified potential transcription factor binding sites within the TGFB3 gene region. Two regions, showing more accessibility in TGFb-treated IMR90 cells, marked as OCR0 (chr14:75968141-75968239) and OCR1 (chr14:75968541-75968896), potentially bind TWIST1/MXI1-FOSL2/AP1 and FOSL2/AP1. A previously described region, OCR2 (chr14:75995418-75995876) contains a SSc-associated SNP (rs56032403) and a TEAD family binding site.

In a series of luciferase reporter assays designed to evaluate gene regulation at the transcriptional level, plasmids containing OCR0, OCR1, and OCR2 regions along with the respective SNPs and point mutations in the predicted transcription factor binding sites were constructed. The assay revealed that OCR0, OCR1, and OCR2 all function as enhancers. Furthermore, mutations at the transcription factor binding sites, as predicted by ChromBPNet, resulted in a reduced enhancer activity of these OCR segments.

These findings not only shed light on the transcriptional regulation of TGFB3 in systemic sclerosis but also identify potential targets for therapeutic intervention, aiming to modulate TGFB3 expression and its consequent fibrotic effects.

Poster 88

Oncogenic gene fusion reprograms methionine metabolism to drive lethal pediatric ependymomas

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Supratentorial ependymomas (ST-EPN) are pediatric brain tumors found in the cerebral hemisphere, driven by the oncogenic fusion of *ZFTA* with *RELA*. Other than surgery and radiotherapy, these tumors do not have effective therapeutic options. Recurrent ST-EPN tumors are highly aggressive and hard to treat, which makes these tumors lethal. To understand the disease mechanism and potentially identify or develop therapeutic targets, we established patient-derived models and interrogated the genetic and metabolic dependencies using cutting-edge, near-genome-wide genetic screening and metabolomic tools. Our integrative transcriptomics and metabolic analysis identify that the ZFTA-RELA fusion drives methionine metabolic reprogramming in the cancer stem cells to regulate permissive epigenetic status and pyrimidine nucleotide synthesis to fuel tumor growth. Limiting methionine or inhibiting MAT2A, a rate-limiting enzyme of methionine metabolism in ST-EPN cells, results in profound cell cycle arrest and senescence compared to other pediatric brain tumors and normal neural stem cells. Methionine consumption, catabolism, and its recycling were all selectively tuned in ZFTA-RELA ependymoma to support cancer stem cell growth profoundly. *In vitro* and *in vivo* metabolic and functional studies further underscore that ZFTA-RELA tumor cells accumulate the metabolic intermediates of pyrimidine nucleotide synthesis through enhanced MAT2A activity and that targeting MAT2A alone or in combination with pyrimidine metabolism inhibitors should reduce the tumor burden and enhance patient survival.

Poster 89

Exploiting a xenotopic tool (LbNOX) to study the impact of tissue specific manipulations of NAD metabolism on aging.

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Nicotinamide adenine dinucleotide (NAD) in cells is found in both oxidized (NAD^+) and reduced (NADH) forms and represents a key NADH/NAD^+ redox pair. In addition, it also serves as an essential substrate for non-redox NAD^+-dependent enzymes, including sirtuins, CD38, and poly (ADP-ribose) polymerases. Multiple studies in different organisms have demonstrated a decline in the total levels of NAD^+ with age in a tissue-specific manner and has led to the hypothesis that a decline in NAD^+ level with age is one of the major contributing factors to the aging process. Multiple pre-clinical studies in rodents provide evidence for the beneficial effects of supplementation with NAD biosynthetic precursors that boost total cellular NAD levels. However, supplementation studies neither allow testing of the tissue-specific roles of restoring NAD^+ levels nor investigate how NADH/NAD^+ ratio itself regulates the aging process, representing a critical knowledge gap. We hypothesize that direct manipulation of the NADH/NAD^+ ratio in a tissue-specific manner is sufficient to recapitulate the effects observed with NAD precursors supplementation on the aging process. In collaboration with Dr. Cracan (Scintillon Institute, USA), who introduced a genetically-encoded tool (LbNOX) to manipulate cellular NADH/NAD^+ ratio, we tested our hypothesis using *Drosophila* as a model system. Our preliminary results demonstrate that LbNOX expression is capable of altering NAD^+ levels and can rescue neuronal cell death induced by reductive stress in the *Drosophila* eye. Further, we have characterized the effects of LbNOX expression, and our results show that ubiquitous expression of LbNOX prolongs lifespan in both females as well as males, promotes resistance towards oxidative stress, and manipulates sleep parameters. We have quantified the levels of NAD in flies to study the changes in NAD levels with age, starvation stress and oxidative stress. We aim to express LbNOX in a tissue-specific manner (particularly in neurons, muscles, and fat body) to dissect its impact at the organismal level.

Poster 90

Differential compounding effects of urban heat island and severe heat wave on growth of diploid and polyploid Duckweed species (Lemnaceae)

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Weedy plant species are incredibly successful in urban environments, exhibiting often extreme tolerance to urban stressors. The urban heat island (UHI) can push these plants to their thermal limits, however, extreme climatic events such as heat waves may exceed these limits. Conversely, polyploid plants typically have greater stress tolerance than their diploid counterparts due to various somatic changes, including greater biomass, water storage organs, and heat shock resistance genes. In this study, we compare the response of two cosmopolitan duckweed species, *Spirodela polyrhiza* and *Lemna minor* to simulated heatwave conditions. Duckweeds are the smallest extant plant species, have a free-floating aquatic growth habit, are commonly found at the surfaces of slow-moving ponds and lakes, and are deployed in urban wastewater treatment and phytoremediation. For this study, three lineages of each species were used, each consisting of two cytotypes for *S. polyrhiza* (2X and 4X), and three cytotypes for *Lemna minor* (2X, 3X, and 4X), with four replicates each. Plants were grown in common garden microcosms with daytime temperatures of 25 °C (optimal growth) and 33 °C (UHI) for three weeks. Microcosms were then divided in half, such that 50% remained in common garden conditions (control) and 50% were exposed to simulated heatwave temperatures for three days (40 °C; n_{Total}=240). Preliminary results indicate that there are no significant differences in growth rate due to UHI alone, and both duckweed species responded similarly regardless of lineage.

Poster 91

Identification of Lysosomal Lipolysis as a Non-canonical Mediator of Adipocyte Fasting- and Cold-induced Lipolysis

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Adipose tissue is a primary storage site for neutral lipids, which are broken down via lipolysis, a metabolic process that occurs in the cytoplasm. Cytoplasmic lipolysis initiated by adipose triglyceride lipase (ATGL), which is widely considered the predominant mechanism of liberating free fatty acids (FFAs) and glycerol from triglycerides during fasting or cold-induced nonshivering thermogenesis. However, the current dogma does not address the fact that lipolysis is still observed in cytoplasmic lipolysis-inactive mice, implying an alternative or complementary pathway exists for processing lipid degradation. We hypothesized that lysosomal lipolysis, mediated by lysosomal acid lipase (LAL), is an alternative pathway that could contribute to adipose lipolysis. We found that LAL expression increases in the adipose tissue of mice in response to fasting, cold exposure, and treatment with CL316,243 (CL), a mimic of cold-induced lipolysis. Similar results were obtained in cultured adipocytes with nutrient-depletion or isoproterenol treatment, mimicking fasting or cold, respectively. We next developed genetically adipose-specific LAL knockout (A-LAL KO) mice, which exhibited lower plasma FFAs when challenged with fasting, cold exposure, or CL treatment. Although no alteration was observed at baseline, oxygen consumption and thermogenesis were significantly impaired in A-LAL KO mice exposed to cold or treated with CL, relative to control mice. Similarly, treatment of C57BL/6J mice with the LAL-specific inhibitor Lalistat (Lali) impaired thermogenesis, as evidenced by suppression of cold- and fasting-mediated upregulation in plasma FFA levels during cold exposure or CL treatment. Lipolysis was inhibited by Lali treatment in cultured adipocytes and fresh adipose tissue of C57BL/6J mice under fasting- or cold-mimic conditions. Furthermore, LAL deficiency did not suppress the adipose expression of ATGL or its cofactors. Overall, our data shows that adipose LAL is an ATGL-independent lipase that likely plays an important role in fasting- and cold-induced lipolysis.

Poster 130

Reconstructing Blood Flow in Data-Poor Regimes Using Low-Rank Approximation

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Transcranial Doppler ultrasound (TCD) is a bedside clinical tool commonly used to measure blood velocity waveforms. However, it provides limited measurements due to wave absorption by skull thickness and probe placement. The limited availability of data can lead to challenges in training machine learning surrogate models, such as deep neural networks or Gaussian process regression, and cause overfitting issues. Therefore, developing a computational model capable of providing predictions or field reconstruction based on sparse data is essential. To address this, we propose a Gaussian Process regression approach based on physics-informed kernels, which provides spatiotemporal and vessel-to-vessel correlations, allowing for blood flow reconstruction in vessels that lack direct measurements. The kernel is constructed by running stochastic one-dimensional blood flow simulations, where the stochasticity captures physiological variability, such as the lack of knowledge about boundary conditions. We demonstrate the performance of the model on two test cases: the abdominal aorta and brain vasculature.

Poster 92

Role of KDM5D in cell proliferation and CHK1 inhibitor sensitivity: implication in prostate cancer therapy

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Epigenetic regulation plays an important role in prostate cancer progression and metastasis. KDM5D is a male-specific histone-modifying enzyme encoded on the Y chromosome that demethylates di- and tri- methylated forms of lysine 4 in histone H3, resulting in transcriptional repression of certain genes. It has been demonstrated that different prostate cancer cell lines exhibit various resistance to an inhibitor targeting the G2/M cell cycle checkpoint kinases CHK1 (SRA737). In our study, we re-examined the KDM5D expressions in these prostate cancer cell lines that are sensitive or resistant to SRA737, investigated the functional role of KDM5D in the proliferation of these cell lines and their potential resistance to SRA737.

Our preliminary results showed that the proliferation and growth of androgen-sensitive line LNCaP were affected negatively to a great extent by knocking down KDM5D expression. The loss of KDM5D expression did not cause significant cell death in another androgen-sensitive line LAPC4 or a castration resistant (CRPC) line 22RV1. Furthermore, increased resistance to a CHK1 inhibitor SRA737 and an ATR inhibitor VE-822 was observed in both LAPC4 and 22RV1. Further experiments demonstrated a self-regulatory cycle between CHK1 and KDM5D, where depletion of KMD5D reduced CHK1 mRNA, while decreased CHK1 caused upregulation of KDM5D. Overexpression of KDM5D using lentiviral vectors in another CRPC line C4-2B that is resistant to SRA737 resulted in increased production of CHK1. The relevance of this regulatory mechanism to CHK1 inhibitor sensitivity is currently under investigation, along with several potential mediators of the differential responses to these inhibitors. Additional CHK1 inhibitors will also be examined in this context. This study may uncover novel biomarkers for CHK1 inhibitors that are currently in clinical trials for various cancers, and KDM5D may be used to stratify prostate cancer patients receiving these highly potent anticancer agents.

Poster 93

A neurodevelopmental epigenetic programme mediated by SMARCD3–DAB1–Reelin signalling is hijacked to promote medulloblastoma metastasis

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How abnormal neurodevelopment relates to the tumour aggressiveness of medulloblastoma (MB), the most common type of embryonal tumour, remains elusive. Here we uncover a neurodevelopmental epigenomic programme that is hijacked to induce MB metastatic dissemination. Unsupervised analyses of integrated publicly available datasets with our newly generated data reveal that SMARCD3 (also known as BAF60C) regulates Disabled 1 (DAB1)-mediated Reelin signalling in Purkinje cell migration and MB metastasis by orchestrating cis-regulatory elements at the DAB1 locus. We further identify that a core set of transcription factors, enhancer of zeste homologue 2 (EZH2) and nuclear factor I X (NFIX), coordinates with the cis-regulatory elements at the SMARCD3 locus to form a chromatin hub to control SMARCD3 expression in the developing cerebellum and in metastatic MB. Increased SMARCD3 expression activates Reelin–DAB1-mediated Src kinase signalling, which results in a MB response to Src inhibition. These data deepen our understanding of how neurodevelopmental programming influences disease progression and provide a potential therapeutic option for patients with MB.

Poster 113

Relationship between fear-avoidance behavior, clinical outcomes, and recovery time in adults following concussion

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Purpose: The purpose of this study was to examine the relationship between fear-avoidance behavior, clinical outcomes, and recovery time in adults following concussion.

Methods: This prospective study included patients aged 18-50 years who presented to a specialty clinic 5-30 days post-concussion. Participants completed a clinical intake (e.g., demographics/medical history), multidomain clinical assessment (Clinical Profile Screen [CP-Screen], Immediate Post-concussion Cognitive Testing [ImPACT], Vestibular/Ocular Motor Screen [VOMS], Patient Health Questionnaire-9 [PHQ-9], Generalized Anxiety Disorder-7 [GAD-7]), and the Fear-Avoidance Components Scale (FACS). Recovery time was ascertained at a subsequent visit(s). Fear-avoidant (FA) and non-fear-avoidant (NFA) groups were classified using cutoffs from the FACS (FA=41-100; NFA=0-40) and compared using independent samples t-tests, X^2 tests, and analyses of covariance.

Results: Seventy-two participants (M=28.7±8.5 years, 69.4% female) were included: 37 (51.4%) in the FA and 35 (48.6%) in the NFA group. Groups did not differ on demographics, medical history, injury characteristics, or CP-Screen symptoms. A greater proportion of the FA group had the anxiety/mood profile (62.2% vs 31.4%, p<0.01). The FA group had worse ImPACT reaction time (t(68)=-2.7, p<0.01, d=-0.7), VOMS visual motion sensitivity (t(67)=-2.3, p=0.03, d=-0.5), GAD-7 (t(70)=-3.9, p<0.01, d=-0.9), and PHQ-9 (t(69)=-2.8, p<0.01, d=-0.7) scores, and longer recovery (t(24)=-2.8, p=0.01, d=-0.9), even when controlling for age and time to clinic (F[1, 22]=4.6, p=0.04, η_p^2=0.17).

Conclusions: Adults who reported high fear-avoidance (e.g., avoiding activity, catastrophizing) had worse clinical outcomes and longer recovery following concussion compared to non-fear-avoidant adults, despite no differences in demographics, medical history, injury characteristics, or symptoms. Clinicians should screen for and counsel adults post-concussion against engaging in fear-avoidance behaviors to improve recovery outcomes.

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