Jefferson College of Life Sciences, Office of Postdoctoral Affairs, and Jefferson Postdoctoral Association

PRESENT

EIGHTEENTH POSTDOCTORAL RESEARCH SYMPOSIUM

SEPTEMBER 19, 2024

Thomas Jefferson University

KEYNOTE SPEAKER

Shruti Naik, PhD

Associate Professor, Immunology and Dermatology
Director, Tissue Repair Program
Ichan School of Medicine at Mt. Sinai

Shruti Naik, PhD, is an international leader in immunology and tissue stem cell biology. She is an Associate Professor in Immunology and Dermatology and the Director of the Tissue Repair Program at Ichan School of Medicine at Mt. Sinai.

Dr. Naik's research uses advanced technologies to study how immune cells communicate with tissues, aiming to develop therapies that stop inflammatory damage and rejuvenate organs at the cellular and molecular levels. She has made groundbreaking discoveries on the microbiota's role in immunity, epigenetic memory in stem cells, and the mechanisms of immune-tissue interactions.

A strong advocate for diversity in science, Dr. Naik has received numerous accolades, including the Regeneron Award for Creative Innovation, L'Oréal For Women in Science Award, Damon Runyon Dale F. Frey Award for Breakthrough Scientist, Blavatnik Award for Young Scientists, Takeda Innovators in Science Award, Pew-Stewart Scholar, NIH Director's Innovator Award DP2, Packard Fellow, Burroughs Wellcome PATH Award, and is a NYSCF Robertson Stem Cell Investigator.

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ACKNOWLEDGEMENTS

The Jefferson Postdoctoral Research Symposium (PRS) is a combined effort of many people. The 2024 PRS Organizing Committee would like to thank:

- Lisa Kozlowski, PhD, Associate Dean, JCLS, for her twenty-plus years of support, concern, and advocacy for Jefferson postdoctoral fellows and postdoctoral mentors
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- Arijita Ghosh, PhD, and Casey Stefanski, PhD, for their contribution as oral presentation session moderators
- Our faculty judges for so willingly giving their time
- The mentors, students, and staff who support the postdocs every day

18TH POSTDOCTORAL RESEARCH SYMPOSIUM

Thursday, September 19, 2024

PROGRAM AGENDA

1st Floor Lobby & Connelly Auditorium, Hamilton Building

10:00-10:45	Poster Set-Up
11:00-12:00	Research Lightning Presentations
12:00-12:30	Lunch Break
12:30-1:50	Poster Presentations and Judging
1:50-2:00	Break
2:00-3:30	Research In-Depth Presentations
3:30-4:00	Refreshment Break
4:00-5:00	**KEYNOTE ADDRESS**

Shruti Naik, PhD
Associate Professor, Immunology and Dermatology
Director, Tissue Repair Program
Icahn School of Medicine at Mt. Sinai

5:00-5:30 Awards Ceremony

DISTINGUISHED MENTOR AWARD

Deepak Deshpande, DVM, PhD
Professor, Department of Medicine, Division of Pulmonary, Allergy & Critical Care Medicine
Director, Center for Translational Medicine

Presented by: Lisa Kozlowski, PhD, Associate Dean, JCLS

POSTDOC PRESENTATION AWARDS

Presented by: Lisa Kozlowski, PhD, Associate Dean, JCLS, and 2024 PRS Organizing Committee Members Benjamin Cartes-Saavedra, PhD, and Shamaila Zafar, PharmD, PhD

2024 POSTDOCTORAL RESEARCH SYMPOSIUM ORGANIZING COMMITTEE

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Chazmyn Riley, PhD
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Advisor

JEFFERSON POSTDOCTORAL ASSOCIATION

Jefferson Postdoctoral Association's (JPA) ultimate goal is to provide postdoctoral fellows with support to learn about the different professional paths that may fit their interests through a series of professional scientific training during the year in addition to creating friendly networking opportunities through multiple social events.

Academic and Professional Activities

- Postdoc Scientific Editing and Reviewing Team (PSERT)
- Postdoctoral Fellowship Application Program (PFAP)
- Technical Skills Seminar Series (TSSS)
- President's Talk (annual lecture by former JPA president)
- Postdoctoral Research Symposium (PRS)

Social Events

- National Postdoc Appreciation Week (NPAW) events
- Around the World event
- Summer BBQ

To continue improving the postdoctoral experience, we need your ideas, involvement and support! JPA offers opportunities to enhance your professional development skill sets:

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- Time management
- Marketing
- Networking and making valuable contacts
- Communication
- Event planning
- Budgeting

For more information and to be part of the community, please contact jpa@jefferson.edu.

2024 DISTINGUISHED MENTOR AWARD NOMINEES

David Abraham, PhD
Professor, Department of Microbiology and Immunology
Associate Dean, Academic Affairs, Sidney Kimmel Medical College

Deepak Deshpande, DVM, PhD
Professor, Department of Medicine, Division of Pulmonary, Allergy & Critical Care Medicine
Director, Center for Translational Medicine

Lucia Languino, PhD
Professor, Department of Pharmacology, Physiology & Cancer Biology

Vittorio Maio, PharmD, MS, MSPH
Professor and Director, Applied Health Economics and Outcomes Research Program
Director, Health Economics and Outcomes Research Fellowship Program

Zheng Ruan, PhD
Assistant Professor, Department of Biochemistry & Molecular Biology

Amit Srivastava, PhD
Assistant Professor, Department of Medicine Cardeza Foundation for Hematologic Research

Jianxin Sun, PhD
Professor, Department of Medicine, Division of Cardiology
Associate Director, Center for Translational Medicine
Director, Vascular Biology and Therapeutics Program

Ryan Tomlinson, PhD
Associate Professor, Department of Orthopaedic Surgery and Department of Oral and Maxillofacial Surgery

Davide Trotti, PhD
Professor, Department of Neuroscience
Director of Research, Weinberg ALS Center
Vickie and Jack Farber Institute for Neuroscience

Mudit Tyagi, PhD, PGDBT
Associate Professor, Center for Translational Medicine

2024 DISTINGUISHED MENTOR AWARD WINNER
Deepak Deshpande, DVM, PhD

The Jefferson Postdoctoral Association and the Office of Postdoctoral Affairs established the Distinguished Mentor Award (DMA) to recognize the commitment and effort of Jefferson faculty members to the mentorship of postdoctoral fellows. This year's winner is Deepak Deshpande, PhD, from the Department of Medicine, Division of Pulmonary, Allergy & Critical Care Medicine, where he is Professor and Director of the Center for Translational Medicine (CTM).

He received a BVSc and MVSc from the Veterinary College, Hebbal, in Bangalore, India. He did his doctoral research at the University of Minnesota, where he obtained his PhD in Molecular Veterinary Biosciences. He was a postdoctoral fellow at Wake Forest University Health Sciences under the mentorship of Dr. Raymond Penn. He has held faculty appointments in India and at the University of Maryland in Baltimore. He moved to Jefferson as an Associate Professor in the CTM and is now Professor and Director of the CTM. His research focuses on G protein-coupled receptors, airway smooth muscle, asthma, pharmacology of airway diseases, and non-coding RNAs in lung diseases.

He was nominated by postdocs who work directly for him and who have him as a co-mentor. One nominator commented that "he is one of the most intellectual people I have had the privilege of learning from and he has a unique way of breaking down and explaining scientific concepts." He helped one postdoc to "become more confident in myself, by trusting my data even though it did not support our hypothesis." Another nominator commented that "he has cultivated a friendly lab environment with positive motivation." All nominators agreed that "we all feel extremely comfortable in approaching him...with any scientific or personal problems." His nominators have had in-depth talks with him about career options and "discussed ways to achieve these career goals, including by introducing us to his network." One nominator wrote that he is a "great sounding board and truly every meeting with him makes you feel enlightened." Another nominator commented that "it is easy for a PI to mentor their own lab personnel, but it truly takes a great mentor to do that for personnel from the entire department." For all these reasons, Dr. Deepak Deshpande was chosen as this year's recipient of the DMA.

Research Lightning Presentations

CONNELLY AUDITORIUM, HAMILTON | 11:00AM — 12:00PM

Moderator: Casey Stefanski, PhD

11:00 - 11:05	Lisa Kozlowski	Opening Remarks
11:05 - 11:13	Sabrine Ben Hamed	Toward the Development of a Pan-Lyssavirus Vaccine
11:13 - 11:21	Ali Calderon-Aparicio	S6K1 controls DNA damage signaling modulated by the MRN complex to induce radioresistance in lung cancer
11:21 - 11:29	Joice Thomas Gavali	Promotion of cardiac fibrosis and heart failure by LMCD1
11:29 - 11:37	Ateesha Negi	Two-Read Sequencing Approach for Decoding Genome-Wide tRNA Modification
11:37 - 11:45	Stepan Nersisyan	Differential abundance of small RNAs and mRNAs in the brains of schizophrenia and bipolar disorder patients
11:45 - 11:53	Mehnoosh Torkzaban	GMP manufacturing of GUCY2C-directed CAR-T cell therapy for metastatic colorectal cancer
11:53 - 12:00	Casey Stefanski	Closing Remarks

03 Toward the Development of a Pan-Lyssavirus Vaccine

Ben Hamed S, Myers J, Chandwani A, Wirblich C, Kurup D, Paran N, Schnell M

Department of Microbiology and Immunology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA

In addition to the rabies virus (RABV), 16 more lyssavirus species have been identified worldwide, causing a disease similar to RABV. Non-rabies-related human deaths have been described, but the number of cases is unknown, and the potential of such lyssaviruses causing human disease is unpredictable. The current rabies vaccine does not protect against divergent lyssaviruses such as Mokola virus (MOKV) or Lagos bat virus (LBV). Thus, a more broad pan-lyssavirus vaccine is needed. Here, we evaluate a novel lyssavirus vaccine with an attenuated RABV vector harboring a chimeric RABV glycoprotein (G) in which the antigenic site I of MOKV replaces the authentic site of rabies virus (RABVG-cAS1). The recombinant vaccine was utilized to immunize mice and analyze the immune response compared to homologous vaccines. Our findings indicate that the vaccine RABVG-cAS1 was immunogenic and induced high antibody titers against both RABVG and MOKVG. Challenge studies with different lyssaviruses showed that replacing a single antigenic site of RABV G with the corresponding site of MOKV G provides a significant improvement over the homologous RABV vaccine and protects against RABV, Irkut virus (IRKV), and MOKV. This strategy of epitope chimerization paves the way towards a pan-lyssavirus vaccine to safely combat the diseases caused by these viruses.

07 S6K1 controls DNA damage signaling modulated by the MRN complex to induce radioresistance in lung cancer

Calderon-Aparicio A, Simone NL

Department of Radiation Oncology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA

Purpose: Radiation is a mainstay for lung cancer treatment, the deadliest tumor worldwide. However, resistance frequently develops, making the identification of new radiosensitizer targets an urgent need in clinical therapy. S6K1, a downstream kinase of mTOR, induces resistance to genotoxic treatments such as radiation, but the mechanisms involved are unknown. We hypothesize that S6K1 could inhibit DNA damage to promote radioresistance. Therefore, we aimed to investigate whether S6K1 inhibition increases the radiosensitivity of lung cancer cells by modulating DNA repair signaling. Methods: Colony formation was determined by clonogenic assays before and after radiation. Protein expression was evaluated by Western blotting. Genetic deletion of S6K1-/- was performed by Crispr-cas9 technology. Results: The radioresistance levels of lung cancer cells positively associated with higher levels of S6K1 signaling activation. Further, pharmacological and genetic targeting of S6K1 sensitized A549 and H661 cells to low doses of radiation compared to controls ($p \leq 0.01$). Next, we studied the activation of the MRN complex, a key activator of DNA repair signaling for radiation-induced damage. The phospho-activation and expression of p-RAD50 and MRE11, MRN complex members, were decreased in most intrinsically radioresistant cells A549 compared to sensitive cells. We found lower levels of p-ATM, a target of the MRN complex, in radioresistant cells, which led to a lower expression of H2AX-γ after radiation. Additionally, the genetic deletion of S6K1-/- increased the levels of p-RAD50, p-ATM and MER11, showing that S6K1 regulates DNA damage by inactivating MRN complex signaling. Conclusions: We showed for the first time that S6K1 inhibition sensitizes lung cancer cells to radiation by decreasing MRN complex-regulated DNA repair signaling. Here, S6K1 could inhibit DNA damage and induce genetic abnormalities accumulation to increase malignity and radioresistance. Our data lay the foundation for more in-depth studies evaluating S6K1 inhibitors as radiosensitizers in cancer.

10 Promotion of cardiac fibrosis and heart failure by LMCD1

Gavali J, Qin Q, Tongmuang N, Summer R, Sun J

Center for Translational Medicine, Department of Medicine, Thomas Jefferson University, Philadelphia, PA

Introduction: Cardiac fibrosis (CF) is a typical pathophysiological process associated with excess ECM deposition, resulting in tissue scaring and organ dysfunction. No specific antifibrotic therapies exist for CF, mainly because of a limited understanding of disease mechanisms. LMCD1 is a member of the LIM family, which is highly expressed in the heart, and its expression is markedly reduced in HF patients. The role of LMCD1 in CF remains poorly understood. The purpose of this study is to define its role in CF and the molecular mechanisms involved. Methods: The expression of LMCD1 in cardiomyocytes and fibroblasts from adult mouse hearts was determined by western blot. TGF-β1 was used to induce CF in vitro in human CFs. A loss-of-functional approach was used to determine the functional significance of LMCD1 in CF using lentivirus-bearing LMCD1 shRNA. Global LMCD1 KO mice were utilized to determine the functional significance of LMCD1 in CF and remodeling in vivo using a murine model of AngII. Echocardiography was performed to measure the cardiac function. Results: LMCD1 is highly expressed in human CFs, and its expression is markedly upregulated in a time and dose-dependent manner in response to TGF-β1. Furthermore, we found that LMCD1 expression was markedly increased in mice hearts after Ang II infusion. In human CFs, LMCD1 KO significantly prevented TGF-β1-induced expression of fibrotic genes. Accordingly, LMCD1 KO mice are protected from developing cardiac fibrosis induced by Ang II infusion. Protein and mRNA levels of fibrotic genes were significantly reduced in the heart of LMCD1 KO mice. Ang II infusion significantly increased LVAW and LVPW in WT mice, which were markedly

attenuated in LMCD1 KO mice. Mechanistically, we found that TGF-β1-induced phosphorylation levels of SMAD2/3 were substantially decreased in LMCD1-deficient CFs. Conclusion: Our findings identified LMCD1 as a critical regulator in promoting CF by targeting TGF-β1/SMAD signaling and suggested that targeted inhibiting LMCD1 may represent a novel therapeutic strategy to ameliorate CF.

21 Two-Read Sequencing Approach for Decoding Genome-Wide tRNA Modification

Negi A, Gamper H, Hou YM

Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA

Genome-wide tRNA molecules decode the genetic code, but their interpretation is not fully understood due to complex post-transcriptional modifications. Mapping these modifications in human tRNAs is particularly challenging due to several factors: the high modification density (15-20%) compared to mRNAs (<0.1%), the diverse and complex chemical structures of these modifications, and their clustering within the stable, compact L-shaped tRNA structure. Traditional RT-based Illumina sequencing has difficulty reading genome-wide tRNA sequences due to these complexities. To overcome these challenges, we propose using 3Dpol, an RNA-dependent RNA polymerase from poliovirus, as an enzymatic reader for 2-read sequencing of tRNAs on the nanopore sequencing platform. Preliminary studies have shown that 3Dpol can efficiently read through each tRNA, producing an RNA copy that remains linked to the native strand. This enables a 2-read sequencing approach where each modification is read twice: once in the 3Dpol-copied strand and once in the native strand. This dual reading provides 2-fold redundancy for each modification, enhancing sequencing accuracy and reliability. The 2-read strategy also addresses a significant limitation of nanopore direct sequencing, which often misreads nucleotides adjacent to modifications. Utilizing 3Dpol's high fidelity helps minimize these errors, allowing for more precise and detailed mapping of tRNA modifications. Preliminary results have successfully generated several 3Dpol-copied tRNA molecules, encompassing a wide range of diverse and complex modifications. The potential impact of 3Dpol-mediated 2-read sequencing is substantial for advancing our understanding of genome-wide tRNA dynamics. This approach promises a more accurate and comprehensive view of tRNA modifications, significantly enhancing our knowledge of how tRNAs decode the genetic code and contribute to protein synthesis. Such insights could have broad implications for understanding cellular processes and developing new therapeutic strategies.

22 Differential abundance of small RNAs and mRNAs in the brains of schizophrenia and bipolar disorder patients

Nersisyan S[1], Loher P[1], Nazeraj I[1], Girdhar K[2], Roussos P[2], Rigoutsos I[1]

[1]Computational Medicine Center, Thomas Jefferson University, Philadelphia, PA [2]Icahn School of Medicine at Mount Sinai, NY

Recent large-scale transcriptomic studies showed widespread alterations in gene expression in brain samples of schizophrenia (SCZ) and bipolar disorder (BD) patients. However, no similar studies assessed the differential abundance of small non-coding RNAs (sncRNAs) in SCZ and BD. We sequenced sncRNAs from 170 post-mortem brain samples, including 53 SCZ patients, 40 BD patients, and 77 healthy controls. We used a state-of-the-art analytical pipeline to map the sequenced reads. The brain sncRNAomes are primarily composed of four molecule types: miRNA isoforms (isomiRs; 57.4% of all sncRNAs), tRNA-derived fragments (tRFs; 17.7%), rRNA-derived fragments (rRFs; 14.3%), and Y RNA-derived fragments (yRFs; 8.0%). There are >600 differentially abundant sncRNAs between SCZ patients and healthy controls. Fold changes derived from the BD vs. control comparison are highly correlated with SCZ changes (r=0.93) but are generally weaker, mirroring the previously reported mRNA-seq findings. The direction of change in disease samples significantly varies by sncRNA type: isomiRs are predominantly expressed at higher levels in SCZ and BD, while tRFs, rRFs, and yRFs have lower abundances in SCZ and BD. Using mRNA-seq datasets of the same samples, we showed and independently validated a striking positive correlation between SCZ/BD-induced gene expression changes and healthy brain aging signatures (r=0.79). Such a high correlation strongly suggests accelerated brain aging caused by SCZ and BD. Interestingly, we observed much lower or even negative correlations when conducting the same analysis with different sncRNA molecule types, suggesting independent layers of regulation of coding and small non-coding RNAs. Finally, we identified multiple modules of co-expressed sncRNAs and mRNAs belonging to several critical pathways, including synaptic signaling and synapse organization, axon development, neurogenesis, behavior, immune response, and inflammation.

28 GMP manufacturing of GUCY2C-directed CAR-T cell therapy for metastatic colorectal cancer

Torkzaban M[1], Baybutt TR[1], McCorkell KA[2], Robertson BB[2], Bashir B[1,3,5], Sarraf S[1], Waldman SA[1,5], Snook AE[1,4,5]

[1]Department of Pharmacology, Physiology, and Cancer Biology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA [2]Clinical Laboratory for Cellular Therapy (Transplant and Cellular Therapy Program), Thomas Jefferson University, Philadelphia, PA [3]Department of Medical Oncology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA [4]Department of Microbiology and Immunology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA [5]Sidney Kimmel Comprehensive Cancer Center, Jefferson Health, Philadelphia, PA

Purpose: Globally, colorectal cancer (CRC) is the third most common cause of cancer death. Guanylyl cyclase C (GUCY2C) is a surface protein selectively expressed in intestine and colorectal tumors. We have previously shown that GUCY2C-directed CAR-T cells (GucyCART) eliminate metastatic CRC without toxicity in mouse models. Here, we have established GMP manufacturing of GucyCART and evaluated its production efficiency and antitumor efficacy. Method: The GucyCART gene construct, including single-chain variable fragment derived from antibody clone 5F9, CD8α hinge, CD8α transmembrane domain, and CD28, 4-1BB, and CD3ζ intracellular signaling domains was cloned into a lentiviral transfer plasmid to produce GMP-grade lentiviral vector (LVV). Using the CliniMACS Prodigy, healthy donor leukopaks underwent T-cell enrichment, activation, LVV transduction, expansion, harvest, and cryopreservation. To evaluate production efficiency, T-cell expansion was quantified, final product viability, %CAR expression, and surface markers (CD3, CD4, CD8) were analyzed by flow cytometry and release safety testing was performed. To assess effector function, intracellular cytokine production and extracellular cytokine secretion following incubation with plate-coated GUCY2C protein were measured by flow cytometry and Luminex immunoassay, respectively. GucyCART cytolysis of the GUCY2C-expressing colorectal cancer cell line T84 was evaluated using the xCELLigence real-time impedance analysis platform. Result: T cells were expanded 15-35-fold across different engineering runs. Final GucyCART product showed >75% viability and ~60% CAR expression with stable viability and phenotype analysis after 12 months cryopreserved storage, produced and secreted IFNγ, TNFα, IL-2, and other cytokines upon exposure to the GUCY2C antigen, and effectively lysed T84 cells. Conclusion: GMP GucyCART manufacturing process shows robust reproducibility, CAR expression, and antitumor activity. The safety and efficacy of GucyCART in metastatic colorectal cancer patients will be examined in an upcoming Phase 1 clinical trial.

Early Discoveries Poster Presentations

1ST FLOOR LOBBY, HAMILTON | 12:30PM — 1:50PM

Sara Beachy	The Impact of Gender, Training Year, Medical Specialty, and the COVID-19 pandemic on Resident Wellness
Pradeep Bhetwal	Survival Analysis of CT Radiomics in Non-Small Cell Lung Cancer Patients
Giulia Calabretto	CD38 Is Up-Regulated In The Most Symptomatic Subgroup Of T-Large Granular Lymphocyte Leukemia (T-LGLL) Patients
Deepu Dowarha	Structural insights into the cell surface proteins Lrp4 and Lrp6
Rupesh Ghimire	AI-based dose prediction for multi-disciplinary lung cancer care using diagnostic PET/CT images
Sowmya Shree Gopal	Cryopreserved Platelet-Derived Extracellular Vesicles Strengthen Endothelial Barrier Integrity
Michael Young	Leveraging a novel tool to generate in vivo mitochondrial DNA deletions and study their regulation
Maite R Zavala	Isoform specific role of actin assembly factor INF2 on cellular calcium dynamics

02 The Impact of Gender, Training Year, Medical Specialty, and the COVID-19 Pandemic on Resident Wellness

Beachy S[1], Luzier J[2], Weisenmuller C[2], Bors K[3], Maurer MA[3], Anees A[4], Lasky T[5], & Calderwood L[6]

[1]Department of Family & Community Medicine, Thomas Jefferson University, Philadelphia PA [2]Department of Behavioral Medicine & Psychiatry, Charleston Area Medical Center, Charleston, WV [3]Department of Family Medicine, Charleston Area Medical Center, Charleston, WV [4]Department of Internal Medicine, Charleston Area Medical Center, Charleston, WV [5]Department of Surgery, Texas Tech University Health Sciences Center, El Paso, TX [6]Center for Health Services and Outcomes Research, Charleston Area Medical Center, Charleston, WV

Data is limited as to which factors most strongly impact medical trainees' well-being, and few studies have assessed trainee's wellness at multiple time points during the COVID-19 pandemic. Additionally, much research has not taken an intersectional approach to understanding the ways in which different facets of a resident's identity impacts their wellness. Thus, more person-centered, probability driven statistical approaches that can produce distinct heterogenous groups of individuals are warranted to understand the way in which different intersecting residents' professional identities are associated with their scores on wellness measures. A latent profile analysis was conducted using institutional data from resident surveys administered from 2019 to 2022 at an academic medical center in West Virginia. There was approximately a 79% response rate for a total of 1,101 surveys completed, and 1,033 were retained for data analysis (i.e., 662 medical residents, 204 surgical residents, 48 pharmacy residents, and 119 residents who did not specify practice type). Per best practices, multiple fit indices and criteria were examined to determine the best fitting model to the data. The 6-class solution was deemed the best fitting which included six distinct heterogenous classes (i.e., Average Majority Pre-COVID, Overall Lack of Wellness, Wellness While Transitioning During COVID, Wellness During Early Medical Residencies, Average Wellness in Females, Doing Well). Broadly, findings indicated that numerous identities (i.e., being female, being in a medical or pharmacy program, and shorter time in program) were associated with lower levels of wellness. Further, findings suggested more disparate levels of wellness pre- and post-COVID in residents with the aforementioned identities. This study should encourage data-driven approaches to wellness initiatives that should be tailored by gender, program type, and year of program.

05 Survival Analysis of CT Radiomics in Non-Small Cell Lung Cancer Patients

Bhetwal P, Choi, W

Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA

Purpose: Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancer cases. Survival analysis is crucial for understanding and predicting outcomes in patients with NSCLC. This work aimed to develop a Cox Proportional Hazards model for survival prediction and compare it with different machine learning approaches like Random Survival Forests and Gradient Boosting. Methods: 422 NSCLC patient CT scans public dataset from The Cancer Imaging Archive (TCIA) including DICOM Radiotherapy Structure Sets, which contain a manual delineation of the 3D volume of the primary gross tumor volume and selected organ-at-risk structures. We preprocessed CT scan image dataset using automated workflow called NSCLC radiomics pipeline. The pipeline involves several critical steps: Data Conversion, Tumor Volume Extraction and Case Matching, Image Resampling and Segmentation, Feature Extraction, and Final Feature Organization. Results: Of 133 features extracted using in-house radiomics tools and 1435 features obtained from PyRadiomics, we retained 1509 radiomics features. We identified 892 robust features using Interclass Correlation Coefficient (ICC) with a threshold greater than 0.9. We synthesized these robust features into 121 representative features using Wilcoxon rank sum tests and agglomerative clustering. We obtained a concordance index of 0.73. Conclusion: We obtained reliable concordance index from diagnostic CT image radiomics and in future, plan to perform survival analysis using longitudinal CBCT radiomics data. The radiomic features from the longitudinal data will contribute to improve prognostic information with enhanced CBCT image quality. Also, the challenge lies in effectively selecting relevant features while filtering out less useful ones. Exploring various dimensionality reduction methods, especially manifold learning techniques, will be crucial. These methods aim to preserve the essential data structure while reducing complexity, potentially enhancing the accuracy and interpretability of survival predictions.

06 CD38 Is Up-Regulated In The Most Symptomatic Subgroup Of T-Large Granular Lymphocyte Leukemia (T-LGLL) Patients

Calabretto G[1,2], Buson E[2], Isabelle C[1], Hutchins Z[1], Scozzafava S[1], Boles A[1], Teramo A[2], Trentin L[2], Semenzato G[2], Zambello R[2], Mishra A[1]

[1]Sidney Kimmel Cancer Center, Department of Medical Oncology, Thomas Jefferson University, Philadelphia, PA [2]Department of Medicine, Hematological Division, Padova University and Veneto Institute of Molecular Medicine (VIMM), Padova, Italy

Please contact the postdoc presenter for information about this abstract.

08 Structural insights into the cell surface proteins Lrp4 and Lrp6

Dowarha, D[1], Ruan, Z[1]

[1]Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA

Please contact the postdoc presenter for information about this abstract.

11 AI-based dose prediction for multi-disciplinary lung cancer care using diagnostic PET/CT images

Ghimire R, Nkwonta L, Bhetwal P, Vinogradskiy Y, Choi W

Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA

Purpose: This study aims to enhance multi-disciplinary clinic for lung cancer treatment by early predicting radiation dose and heart toxicity for patients eligible for stereotactic body radiation therapy (SBRT). Leveraging PET/CT, traditionally used for cancer screening and staging, this approach predicts doses before treatment planning without requiring target volume and organs at risk (OAR) delineation or radiotherapy prescription. This approach could significantly improve clinical decision-making by providing critical information early in the treatment process. Methods: The study included 113 lung cancer patients treated with SBRT who had both diagnostic PET/CT scans and dose map available. Rigid registration of the dose map from the planning CT onto the PET/CT was performed for dose prediction. Three-dimensional convolutional neural network (3D CNN) operations were implemented using two model architectures (3D UNET and 3D Attention UNET) to train prediction models with different input image types: PET/CT, PET only, and CT only. Root Mean Square Error (RMSE) between the predicted and actual distribution in heart volume was evaluated the model performance. Additionally, mean heart dose differences (MHDD) were evaluated to assess the clinical utility of dose prediction. Results: The findings suggest that models from UNET and Attention UNET, accurately predict the dose distribution. The RMSE in UNET and Attention UNET for inputs (PET/CT, CT, PET only) were (2.91 $\pm$ 4.86, 2.84$\pm$ 5.07, 7.89 $\pm$ 3.86) and (2.45 $\pm$ 4.86, 2.64 $\pm$ 5.09, 2.83 $\pm$ 4.73) respectively. MHDD values in UNET and Attention UNET were (0.69 $\pm$ 3.81, 1.11 $\pm$ 3.93, -5.04 $\pm$ 4.23) and (0.90 $\pm$ 3.75, 0.84 $\pm$ 3.92, 0.64 $\pm$ 3.80) respectively. The UNET PET-only model had larger values for both metrics, suggesting the necessity of using PET/CT input while using UNET models. Conclusion: AI-based models can predict dose distributions, providing valuable information before treatment planning. This novel approach could improve clinical decision-making and the treatment process in multi-disciplinary clinics.

13 Cryopreserved Platelet-Derived Extracellular Vesicles Strengthen Endothelial Barrier Integrity

Gopal SS, Kaur M, Srivastava A

Department of Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, Cardeza Foundation for Hematologic Research, Philadelphia, PA

Introduction: Platelet-derived extracellular vesicles (PEVs) effectively control bleeding, maintain vascular integrity, & support endothelial barrier function, offering promising alternative to platelet transfusions. However, PEVs should be isolated from fresh platelets within 5 days. Platelet cryopreservation can extend shelf-life, but efficacy of EVs isolated from cryopreserved platelets is unexplored. This study investigates potential benefits & therapeutic impact of PEVs isolated from cryopreserved platelets compared to fresh platelets on lung endothelial barrier dysfunction. Methods: PEVs were isolated from fresh and cryopreserved platelets, followed by comprehensive characterization. Human pulmonary endothelial cells (HULEC-5a) were pretreated with PEVs (1.47x10^7)/reaction, & subsequently exposed to thrombin (2U/ml) for 1 hour. Endothelial barrier permeability was assessed using transendothelial electrical resistance (TEER) measurements & expression of tight junction proteins were analyzed via Western blot. Endothelial-mediated inflammation was evaluated through RT-PCR and accumulation of reactive oxygen species (ROS) was quantified using flow cytometry. Results: TEM analysis of cryopreserved PEVs revealed uniformly spherical structures with a microvesicle origin (249.1$\pm$3.7 nm), and expressed CD41, CD9, CD63 & CD31 similarly to fresh PEVs. Fresh and cryopreserved PEVs effectively mitigated thrombin-induced barrier permeability. However, cryopreserved PEVs significantly maintain endothelial barrier integrity and enhances expression of tight junction proteins. Cryopreserved PEVs markedly reduced thrombin-induced ROS accumulation and suppressed inflammatory gene expression, suggesting enhanced anti-inflammatory effects. Conclusion: Our study highlights promising therapeutic potential of cryopreserved PEVs which address storage challenges and deliver superior therapeutic benefits compared to fresh PEVs. Cryopreserved PEVs could be attributed as a compelling candidate for future translational studies, offering enhanced treatment outcomes in critical settings.

30 Leveraging a novel tool to generate in vivo mitochondrial DNA deletions and study their regulation

Young MP, Tigano M

Department of Pathology & Genomic Medicine, Thomas Jefferson University, Philadelphia, PA

Single Large-Scale Mitochondrial Deletions Syndromes (SLSMDS) are primary mitochondrial disorders (PMD) driven by loss of functional respiration due to large deletions in mitochondrial DNA (mtDNA). As patients affected by SLSMDSs such as Pearson's Syndrome often succumb at an early age, it is critical to identify novel therapeutic approaches. A barrier to this goal is a lack of tools which model physiological accumulation of mtDNA deletions, a condition known as heteroplasmy. Current models, including patient derived cell lines and a single mouse model, provide a framework for studying SLSMDSs, but are limited by critical several factors: 1) failure to recapitulate earlier stages of disease progression when specific cellular pathways might be driving mutation accumulation; 2) do not provide a dynamic range of heteroplasmy levels but rather a single defined percentage of deletion. Thus, new tools to study mtDNA deletions are urgently needed to identify novel therapeutic approaches for SLSMDS treatment. From the published observation that one double-stranded break delivered at a specific locus of the mitochondrial genome is sufficient to generate a 4977-bp mtDNA deletion, we engineered a new genetic tool termed ΔmtDNAind allowing the inducible and controlled accumulation of mtDNA deletions. This tool is innovative in design and scope as it addresses the shortcomings of current models, including: 1) exhibits dynamic mtDNA deletion accumulation up to and over the pathological threshold; 2) gives ability to study the biological processes driving deletion accumulation. To begin, we will generate ARPE-19 and hESC models stably expressing ΔmtDNAind and characterize the cellular responses triggered by mtDNA deletion accumulation using cutting edge multi-omics single-cell ATAC sequencing. We will then manipulate the perturbed pathways to identify strategies that inhibit the accumulation of deletions. Last, we will leverage the multipotent nature of hESC to model lineages that are either refractory or permissive to deletion accumulation, a distinctive feature of PMDs.

32 Isoform specific role of actin assembly factor INF2 on cellular calcium dynamics

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Timely and regulated actin filament turnover regulates various cellular functions including inter-organelle interaction and homeostasis. INF2

(Inverted formin 2) is a member of the formin family of actin assembly factors that promote both actin nucleation and filament elongation. In cells, INF2 exists in two distinct isomeric forms, INF2-caax and INF2-noncaax. INF2-caax localizes to Endoplasmic Reticulum (ER) while INF2-noncaax exists in the cytosol. Gain-of-function dominant mutation of INF2 has been associated with FSGS and CMTD. Previous reports have shown that INF2-caax generated actin filaments on the ER acutely induces ER-mitochondrional contacts (ERMC) and facilitates ER to mitochondrial calcium transfer that promotes mitochondrial division (PMID: 29142021). While the majority of the cellular role of INF2 has been attributed to mitochondrial dynamics, its role on ER physiology and dynamics have remain elusive. In the present study we present evidence of distinct isoform specific role of INF2 in agonist-induced ER calcium release and store operated calcium entry (SOCE). Both chronic (CRISPER KO) and acute (siRNA/shRNA) depletion of total INF2 results in reduced agonist-induced IP3-mediated ER calcium release in HeLa and MEF cells. INF2 depleted HeLa cells also show an increase in cytosolic calcium oscillations when using sub-maximal dose of histamine. Interestingly SERCA inhibition in HeLa-INF2-KO cells showed reduced ER calcium compared to WT cells indicating defects in store-refiling. Indeed, calcium addition after store depletion showed a significant decrease of SOCE corroborating the reduced stores as seen in HeLa-INF2-KO cells previously. Interestingly, while INF2-noncaax alone could rescue the SOCE defect, both isoforms were required to rescue agonist-induced calcium release. These results indicate that distinct isoforms of INF2 play specific role in both ER Ca2+ release and SOCE. Future experiments will unravel the specific role of these isoforms and the molecular mechanism involved in the processes.

Poster Presentations

1ST FLOOR LOBBY, HAMILTON | 12:30PM — 1:50PM

Lakhikumar Sharma Adhikarimayum	Cocaine-induced DNA-PK relieves RNAP II pausing by promoting TRIM28 phosphorylation
Priyan Bhattacharya	A multiscale network model of tumor microenvironment to predict immunotherapeutic response of head and neck cancers
Yolanda Gomez-Galvez	Handling associated with repeated behavioral testing and high dose of human bone marrow mesenchymal stem cells (hBM-MSCs)-derived extracellular vesicles (EVs) improve post-MCAO recovery in rats
Stephen Hurst	Development of an optogenetic tether to study submitochondrial zoning of the cardiac MCU complex in simplified environments
Arun Kumar Jannu	HDAC11 inhibition reduces airway smooth muscle cell contraction
Sam Sharifzadeh Javidi	Regionally Unconstrained State Transition Energy Consumption in Temporal Lobe Epilepsy
Mandeep Kaur	Histopathological Insights into the Role of Fibrinogen in Amyotrophic Lateral Sclerosis Pathogenesis
Pauline Michel-Flutot	PTEN inhibition promotes robust growth of bulbospinal respiratory axons and partial recovery of diaphragm function in a chronic model of cervical contusion spinal cord injury
Sarah Newton	Investigating G-Quadruplexes as Major Regulators of TDP-43-mediated Cryptic Splicing
Pranay Ramteke	Sirtuin 6 Is Critical for Maintaining Intervertebral Disc Homeostasis
Victor Hugo Sánchez-Vázquez	Mitochondrial calcium signaling and fusion dynamics are dependent on the disaggregase CLPB
Viren Soni	Development and Evaluation of Buprenorphine Sublingual Formulation for the Treatment of Neonatal Opioid Withdrawal Syndrome
Emily Wakschal	The Association Between Heterosexism, Social Support, and PTSD Symptoms Among Trauma-Exposed Sexual Minority Women
Tian Yuan	NSD2 mutation drives an aberrant epigenetic landscape underlying the relapse of T-cell acute lymphoblastic leukemia
Qirui Zhang	Thalamic Functional Organization Features Distinguish Multiple Key Clinical "Conditions" of Focal TLE

01 Cocaine-induced DNA-PK relieves RNAP II pausing by promoting TRIM28 phosphorylation

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Drug abuse continues to pose a significant challenge in HIV control efforts. In our investigation, we discovered that cocaine augments DNA-PK activation by enhancing its phosphorylation at S2056 and triggers the translocation of DNA-PK into the nucleus. We observe the enhanced DNA-PK recruitment at the HIV long terminal repeat (LTR) following cocaine exposure. By activating and facilitating the nuclear translocation of DNA-PK, cocaine effectively orchestrates multiple stages of HIV transcription, thereby promoting HIV replication. Additionally, our study indicates that cocaine-induced DNA-PK promotes hyper-phosphorylation of RNA polymerase II carboxyl-terminal domain (CTD) at Ser5 and Ser2 sites, enhancing both initiation and elongation phases, respectively, of HIV transcription. Cocaine's enhancement of transcription initiation and elongation is further supported by its activation of cyclin-dependent kinase 7 (CDK7) and subsequent phosphorylation of CDK9, thereby promoting positive transcriptional elongation factor b (P-TEFb) activity. We demonstrate that cocaine, through DNA-PK activation, promotes the specific phosphorylation of TRIM28 at Serine 824. This modification converts TRIM28 from a transcriptional inhibitor to a transactivator for HIV transcription. Additionally, we observe that phosphorylation of TRIM28 (p-TRIM28, S824) promotes the transition from the pausing phase to the elongation phase of HIV transcription, thereby facilitating the production of full-length HIV genomic transcripts. This finding corroborates the observed enhanced RNAP II CTD phosphorylation at Ser2, a marker of transcriptional elongation, following cocaine exposure. Accordingly, upon cocaine exposure, we observed elevated recruitment of p-TRIM28-(S824) at the HIV LTR. Overall, our results have unraveled the intricate molecular mechanisms

underlying cocaine-induced HIV transcription and gene expression. These findings hold promise for the development of highly targeted therapeutics aimed at mitigating the detrimental effects of cocaine in individuals living with HIV.

04 A multiscale network model of tumor microenvironment to predict immunotherapeutic response of head and neck cancers

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Squamous cell carcinoma of the head and neck (HNSCC) is one of the most common cancers, accounting for over 90% of head and neck carcinoma prevalence. Existing therapies targeting the tumor-cell-intrinsic signaling mechanisms underestimate the tumor-promoting role of the non-tumor cells within the microenvironment (TME). Immune checkpoint inhibition (ICI) has emerged as a critical treatment strategy for HNSCC that halts the immune escape of the tumor cells. Despite a few desirable advantages over traditional therapeutic approaches, ICI remains ineffective for some patients. The present work uses a computational modeling framework to decipher the mechanisms behind the survival, growth, and non-response to ICI treatments in HNSCC. We constructed a multicellular TME network with relevant molecular factors mediating cell-cell interactions and cell state transitions. Stability analysis over a wide range of parameter values identified the possible TME subtypes, which, as determined by the molecular markers, are characterized by the presence(absence) of immune and fibroblast cell types such as Immune or Fibro-dominated, Immune, and/or Fibro-deficient phenotypes. Simulation results suggest that in pre-ICI conditions, the growth and proliferation of the tumor cells critically depend on the reinforcing loop between the pro-invasive cancer-associated fibroblasts (CAFs) and the tumor cells, which are protected by the exhausted T cells from the immune response.

Furthermore, our analysis indicates that ICI-based intervention hampers the proliferating effect of the CAF-tumor interaction loop, and the quantitative balance between the CAFs and the cytotoxic T cells governs the post-ICI outcome. Based on these results, we propose that the pre-ICI TME subtype is dominant in determining the post-ICI outcome. The computational predictions closely align with the observations from recent experimental studies and clinical findings. Finally, the model-guided approach enables us to explore TME subtype-specific molecular interventions to improve the efficacy of ICI therapy.

12 Handling associated with repeated behavioral testing and high dose of human bone marrow mesenchymal stem cells (hBM-MSCs)-derived extracellular vesicles (EVs) improve post-MCAO recovery in rats

Gomez-Galvez Y[1,2,3], Gupta M[7], Kaur M[7], Fusco S[5,6], Podda MV[5,6], Grassi C[5,6], Srivastava A[7], Iacovitti L[1,2,3,4], Blanco-Suarez E[1,2,3,4]

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Ischemic stroke, when a clot disrupts the blood supply to the brain, often causes long-term disabilities and death. Removing the clot to allow reperfusion is the standard treatment, but it is effective only in selected patients during a narrow therapeutic window. To improve sensorimotor function, rehabilitation with physical therapy is needed. Bone marrow mesenchymal stem cells (BM-MSCs)-derived extracellular vesicles (EVs) have also been shown to restore brain damage and function, mainly using the intravenous route. This administration route causes EVs to accumulate in peripheral organs, in comparison to the non-invasive intranasal route that allows EVs to reach the brain. This research study aims to understand how intensive handling (in the form of sensorimotor behavioral tests) and/or intranasal EVs, alone or in combination, restore neurological function and ischemic damage in the transient middle cerebral artery occlusion (MCAO) model of stroke in rats. Non-handled rats were evaluated by the modified Neurological Severity Score (mNSS) and Magnetic Resonance Imaging (MRI) at 2, 28, and 56 days post-stroke (dps). Handled rats were exposed to the mNSS, but also other sensorimotor tests such as the beam balance, corner, grid walking, forelimb placement, and cylinder tests, together with MRI at 2, 7, 14, 21, and 28 dps. Non-handled and handled cohorts received either saline (controls) or various intranasal EV treatments: low multidose (8 x 0.8 x 109 EVs, twice/wk for 4 wks), high single dose (2.4 x 109 EVs, at 2 dps), or high multidose (8 x 2.4 x 109 EVs, twice/wk for 4 wks). Animals were sacrificed at 28 or 56 dps, and brains were dissected for postmortem analysis. Our results showed that the mNSS significantly recovered in handled MCAO rats and/or those treated with a high dose of EVs compared to non-handled or low-dose EV-treated rats. This behavioral recovery occurred without affecting ischemic damage. Altogether, this work reveals how intensive behavioral testing and dose/frequency-dependent EV treatment contribute to improved post-stroke functional recovery.

14 Development of an optogenetic tether to study submitochondrial zoning of the cardiac MCU complex in simplified environments

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Background: The heart requires a means to match energetic demand with supply. The prevailing theory is that during high demand related to more intense beating, a part of the Ca2+ released from the SR, which couples the excitation to contraction, is sequestered by mitochondria to activate Ca2+-responsive enzymes of the TCA cycle. In order to prevent short circuits in Ca2+ cycling uncoupled from TCA cycle/ATP production, we observed cardiac mitochondrial Ca2+ transport is spatially subdivided. The MCU complex (MCUc) is preferentially localized to the intake zone adjacent to the junctional (j)SR and the NCLX is maintained in the extrusion zone distal to jSR. In order to identify the molecular mechanism behind this mitochondrial zoning we have developed optogenetic tethers to simulate the localized environment unique to the cardiomyocyte in a simplified cell system that can be easily manipulated at the genetic level.

Using CRISPR-Cas9 we introduced our tether into the AAVS1 safe harbor locus of wildtype or MCU, SMDT1 double-Knockout (MEdKO) Cos7, HeLa, or HEK293T cells The tether connects a calcium-permeable channel rhodopsin (CapCHR2) in the plasma membrane via a rapamycin-dependent dimerization domain (FRB) to its dimerization partner FKBP fused to a mitochondrial outer membrane anchor (AKAP) and a calcium sensor jRCamP1b. To monitor MCUc localization we employed a tri-partite GFP system to identify only those MCUc that are conductive (incorporate both, MCU and EMRE). Results: 1) We demonstrated the tether's correct trafficking, the CapCHR2 calcium conductance and rapamycin-dependent dimerization, using both wide field fluorescent and super resolution microscopy. 2) Using the MEdKO cells rescued with MCU-GFP10 GFP-11-EMRE

and Matrix targeted GFP1-9 we demonstrate specific labeling of the conductive MCU:EMRE complex and verify its functional rescue. Conclusion: This new model enables the study of a cardiac-like mitochondrial calcium environment. The genetic system will also serve as a platform to study both mitochondrial calcium influx and efflux in intact cells.

15 HDAC11 inhibition reduces airway smooth muscle cell contraction

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Aberrant airway smooth muscle (ASM) contraction, typically mediated by Gq-mediated G protein-coupled receptors, is a cardinal feature of asthma. The most commonly used bronchodilators (beta 2 adrenergic receptor agonists) aim to inhibit Gq-mediated signaling in ASM by activating the Gs-mediated signaling pathway to promote ASM relaxation. However, prolonged use of beta-agonists leads to receptor desensitization and waning of therapeutic effect. Our recent findings suggest that HDAC11 inhibition by FT895 is sufficient to relax contracted ASM, and also augments signaling by the beta-agonist Isoproterenol in ASM. Studies using human precision-cut lung slices (hPCLS) demonstrate that FT895 treatment promotes relaxation of contracted airways. Calcium mobilization assay data show that FT895 pretreatment for one hour reduces histamine-induced calcium mobilization in human ASM cells. Western blotting data suggest that FT895 pretreatment regulates the histamine-induced phosphorylation/activation of pro-contractile proteins MLC20 and MYPT1, ultimately leading to the inhibition of ASM cell contraction. HDAC11 inhibition represents a novel strategy to regulate ASM contraction in the management of asthma. Funding: HL058506

16 Regionally Unconstrained State Transition Energy Consumption in Temporal Lobe Epilepsy

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Please contact the postdoc presenter for information about this abstract.

17 Histopathological Insights into the Role of Fibrinogen in Amyotrophic Lateral Sclerosis Pathogenesis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder related to neuroinflammation and neurovascular dysfunction. A fundamental change at neurovascular interface is influx of coagulation factors into central nervous system (CNS) due to increased blood–brain barrier permeability disrupting the homeostasis between vasculature and CNS resulting into inflammation & motor neuron degeneration. The interplay between inflammation and coagulation involving fibrinogen, a glycoprotein converted to fibrin to facilitate clot formation in coagulation cascade, can provide insights to its contribution in neuroinflammation and neurovascular dysfunction. Therefore, this preliminary study addresses the role of fibrinogen in ALS progression and explores gender differences. Methods: Lumbar region of spinal cord from wild-type and ALS mice were collected from male and female mice at pre- (~64 days) and post-symptomatic stage (~130 days) of disease. Tissue was fixed in 4% paraformaldehyde. Cryosections (10µm) were mounted on coated glass slide and used for immunohistochemical analyses using primary antibodies against fibrinogen, Iba1 (microglia activation marker) and NeuN (neuronal marker). Fluorescent intensities were measured and quantified using ImageJ software. Results: We observed increased levels of fibrinogen deposits in spinal cord of both pre- and post-symptomatic ALS mice. Expression of fibrinogen was markedly increased in the post-symptomatic stage of ALS. Infiltration of fibrinogen into ALS spinal cord parenchyma was observed, which could contribute to neurodegeneration in ALS. Expression was more pronounced in the male mice than in the female mice. Moreover, increased inflammation was also confirmed in the ALS mice in comparison to the wild-type mice. Conclusion: Significant increase in fibrinogen levels in spinal cord suggests its accumulation may promote local inflammation and axonal damage, exacerbating the progression of ALS, specifically in male mice. Fibrinogen may be used as a novel blood marker to further investigate ALS.

18 PTEN inhibition promotes robust growth of bulbospinal respiratory axons and partial recovery of diaphragm function in a chronic model of cervical contusion spinal cord injury

Michel-Flutot P[1], Cheng L[1], Thomas SJ[1], Lisi B[1], Schwartz H[1], Lam S[1], Lyttle M[1], Jaffe DA[1], Smith G[2], Li S[2], Wright MC[3], Lepore AC[1]

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High spinal cord injury (SCI) leads to persistent and debilitating compromise in respiratory function. Cervical SCI not only causes the death of phrenic motor neurons (PhMNs) that innervate the diaphragm, but also damages descending respiratory pathways originating in the rostral ventral respiratory group (rVRG) located in the brainstem, resulting in denervation and consequent silencing of spared PhMNs located caudal to injury. It is imperative to determine whether interventions targeting rVRG axon growth and respiratory neural circuit reconnection are efficacious in chronic cervical contusion SCI, given that the vast majority of individuals are chronically-injured and most cases of SCI involve contusion-type damage to the cervical region. We therefore employed a clinically-relevant rat model of chronic cervical hemicontusion to test therapeutic manipulations aimed at reconstructing damaged rVRG-PhMN-diaphragm circuitry to achieve recovery of respiratory function. At a chronic time point post-injury, we systemically administered: an antagonist peptide directed against phosphatase and tensin homolog (PTEN), a central inhibitor of neuron-intrinsic axon growth potential; an antagonist peptide directed against receptor-type protein tyrosine phosphatase sigma (PTPσ), another important negative regulator of axon growth capacity; or a combination of these two peptides. PTEN antagonist peptide (PAP4) promoted partial recovery of diaphragm motor activity out to nine months post-injury, while PTPσ peptide did not impact diaphragm function after cervical SCI. Furthermore, PAP4 promoted robust growth of descending bulbospinal rVRG axons caudal to the injury within the denervated portion of the PhMN pool, while PTPσ peptide did not affect rVRG axon growth at this location that is critical to control of diaphragmatic respiratory function. In conclusion, our non-invasive PAP4 strategy can successfully promote significant regrowth of damaged respiratory neural circuitry and also partial recovery of diaphragm motor function in a preclinical model of chronic cervical contusion.

23 Investigating G-Quadruplexes as Major Regulators of TDP-43-mediated Cryptic Splicing

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Please contact the postdoc presenter for information about this abstract.

25 Sirtuin 6 Is Critical for Maintaining Intervertebral Disc Homeostasis

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Introduction: Intervertebral disc degeneration (IVDD) is one of the main contributors for low back pain viz. the leading cause of disability worldwide. 1. Although environmental and genetic factors are the known etiological causes for disc degeneration, age is still the most significant risk factor. Recent findings report that senescence plays a major role in ageing as well as disc degeneration in human and mouse models. 2. Interestingly, Sirtuin 6 (SIRT6) has been shown to decelerate ageing across different species as well as have an anti-senescence effect. 3. However, its role in intervertebral disc degeneration is largely unknown. Therefore, the aim of this study is to explore the role of SIRT6 in intervertebral disc degeneration. Results: SaF-O staining and Modified Thompson grading suggest that SIRT6cKo mice exhibit significantly higher grades of degeneration as compared to their respective age matched controls. uCT analyses of SIRT6 cKO mice showed significant changes in disc height (DH), vertebral height (VH) and disc height index (DHI) as compared to WT control mice. Surprisingly, Picrosirius red staining showed significant differences in the collagen content. Interestingly, immunohistochemistry analysis showed an increase in p21, a bonafide marker of senescence in SIRT6 cKO discs as compared to their respective controls. Lipofuscin staining and IHC analysis shows changes in DNA damage and autophagy. Increased p21 in SIRT6 cKO mice suggests that senescence- the major etiological factor for disc degeneration, is regulated by SIRT6. Therefore, SIRT6 cKO accelerates disc degeneration via senescence. Significance / Clinical Relevance: In summary, our work provides new insights into SIRT6cKO mediated disc degeneration at the pathological, cellular, and molecular levels, thereby defining the significance of epigenetic landscape in this unique tissue. Further exploration of these findings may lead to development of therapeutic targeting of SIRT6 to mitigate disc degeneration.

26 Mitochondrial calcium signaling and fusion dynamics are dependent on the disaggregase CLPB

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Cells possess protein disaggregases which restore the solubility of the aggregated proteins, thus maintaining the proper protein structure and function. Disruption of the disaggregase activity compromises cell function due to toxic protein aggregation. In metazoan, it has been described disaggregase activity in cytoplasm and nucleus; however, it remains unknown how aggregated proteins are solubilized and reactivated in mitochondria. Recently, Caseinolytic mitochondrial peptidase chaperone subunit B (CLPB), a mitochondria-resident protein linked to human disease, was shown to solubilize aggregated mitochondrial proteins, and the lack of CLPB was associated with insolubility of MICU1 and MICU2, regulators of the mitochondrial calcium uniporter complex (mtCU), and OPA1, a main mediator of mitochondrial fusion; however, the functional outcome remains unclear. Hence, we hypothesized that CLPB might be required to maintain mitochondrial Ca2+ homeostasis and fusion dynamics. To test this, we used WT and CLPB KO HAP1 cells, and performed biochemistry experiments, epifluorescence Ca2+ imaging, fluorometric Ca2+ assays and confocal imaging. Our data shows that the lack of CLPB alters the composition of the mtCU which is linked to reduced mitochondrial Ca2+ uptake and lower resting mitochondrial [Ca2+]. Acute CLPB rescue restored normal mitochondrial Ca2+ signaling. We showed that this effect is specific to mitochondrial Ca2+ homeostasis because the absence of CLPB does not affect cytosolic Ca2+ and mitochondrial membrane potential. Furthermore, we found that absence of CLPB leads to reduced levels of OPA1 and impaired OPA1 proteolytic cleavage of the long forms. These outcomes were accompanied by fusion impairment and an increase in mitochondrial fragmentation. Overall, our findings indicate that mitochondrial Ca2+ homeostasis and fusion activity are dependent on CLPB, and their impairments might contribute to mitochondrial function dysregulation and likely the disease caused by CLPB mutants.

27 Development and Evaluation of Buprenorphine Sublingual Formulation for the Treatment of Neonatal Opioid Withdrawal Syndrome

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The FDA and professional societies recommend that where possible pediatric drug formulations should not contain ethanol. A widely used buprenorphine formulation used in the treatment of neonatal opioid withdrawal syndrome (NOWS) contains 30% ethanol. We first developed an ethanol-free buprenorphine oral solution, and a 60-day stability study was performed evaluating this formulation under both storage settings (2-6°C and 25°C with 60% relative humidity) to assess the drug substance's stability in an ethanol-free environment. A gel formulation has many advantages, it allows for precise dosing via administration of the drug substance as well as, gives the ability for flexible-dose regimens as it can be tailored per patient requirements. This formulation gives ease of administration, as gels are easier to apply to topical surfaces than other formulations. Using a gel sublingually can increase the absorption through the sublingual mucosa and increase the bioavailability of the drug substance. Owing to the advantages of a gel formulation, an oral gel of buprenorphine was pursued using different polymers as gelling agents, and hydroxypropyl methylcellulose (HPMC) was determined as the ideal candidate for this formulation. This formulation was characterized by establishing the stability and efficacy of the oral gel by performing a 30-day stability study and exploring the potential clinical benefits and feasibility of implementing this formulation in neonatal care settings to improve outcomes for neonates affected by NOWS and pave the way for further advancements in pediatric formulations.

29 The Association Between Heterosexism, Social Support, and PTSD Symptoms Among Trauma-Exposed Sexual Minority Women

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Background: Sexual minority women are at increased risk of trauma exposure and subsequent diagnosis of posttraumatic stress disorder (PTSD) as compared to their heterosexual counterparts. According to minority stress theory, the mechanisms through which this disparity exists may be due in part to both minority and non-minority specific factors. Exposure to daily heterosexism, or the societal disapproval of non-heterosexual identity, is associated with heightened PTSD symptoms among this population. Further, internalized heterosexism and posttraumatic cognitions have both been identified as uniquely predictive in the association between daily heterosexism and PTSD symptom severity (Dworkin et al., 2018). Evidence shows that social support may buffer the impact of these minority and non-minority specific stressors on adverse mental health outcomes. This study aims to (1) explore the association between daily heterosexist experiences and PTSD symptom severity, (2) evaluate the indirect associations between daily heterosexist experiences and PTSD symptoms via both internalized heterosexism and posttraumatic cognitions, and (3) evaluate social support as a moderator of the indirect associations between daily heterosexist experiences and PTSD via internalized heterosexism and posttraumatic cognitions. Methods: Data were obtained from 303 trauma-exposed sexual minority women, age 18-79 via the survey hosting site Prolific. Hypotheses were tested through parallel mediational and moderated mediational linear regression modeling. Results: There was a significant indirect association between daily heterosexist experiences and PTSD symptoms via posttraumatic cognitions. Social support significantly moderated the indirect association between daily heterosexist experiences and internalized heterosexism, and the association between daily heterosexist experiences and posttraumatic cognitions. Conclusion: These findings highlight contributions of minority stress on both minority and non- minority specific processes, and the complex role of social support on these associations.

31 NSD2 mutation drives an aberrant epigenetic landscape underlying the relapse of T-cell acute lymphoblastic leukemia

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Background: NSD2 (nuclear receptor binding SET domain protein 2) is a histone methyltransferase specific for H3K36me2. Recurrent NSD2 mutations are identified in relapsed T-cell acute lymphoblastic leukemia (T-ALL). NSD2 p.E1099K mutation affects gene expression through an increase of H3K36me2 and a decrease of H3K27me3. However, the role of NSD2 mutation in T-ALL is still unclear. Aim: To elucidate the epigenetic landscape driven by NSD2 mutation and evaluate the efficacy of a novel NSD2 inhibitor in T-ALL. Methods: We generated isogenic T-ALL cell lines with or without NSD2 mutation using CRISPR/Cas9 gene editing. We performed in vivo experiments to observe tumor burden, leukemia cell infiltration, and survival of the NOD/SCID mice. We determined RNA-Seq, ATAC-Seq, and ChIP-Seq. Finally, we evaluate the efficacy of a novel NSD2 inhibitor. Results: The NOD/SCID mice xenografted with NSD2 mutant cells developed significantly higher tumor burden and CNS infiltration. RNA-Seq and ATAC-Seq analysis showed that reversion of NSD2 mutation to WT caused more closed chromatin and downregulated gene expression while insertion of NSD2 mutation to WT cells led to more open chromatin and upregulated gene expression. Most of the upregulated genes correlated with neural development and adhesion, contributing to CNS infiltration. The downregulated NR3C1 gene was associated with GC resistance. The genes upregulated by NSD2 mutation lost H3K27me3 at the promoters but gained H3K36me2 at the promoters and the whole gene body. Conversely, the downregulated genes gained H3K27me3 and lost H3K36me2 in their promoters. A novel NSD2 inhibitor remarkably reduced H3K36me2, increased H3K27me3, inhibited leukemic cell growth, and induced apoptosis. Conclusions: NSD2 mutation drives an oncogenic epigenetic landscape underlying CNS infiltration and GC resistance, contributing to T-ALL relapse. A novel NSD2 inhibitor can reverse the aberrant histone modification and inhibitor T-ALL cell growth.

33 Thalamic Functional Organization Features Distinguish Multiple Key Clinical "Conditions" of Focal TLE

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Purpose: Numerous dichotomous clinical features have been studied to identify the core, unique neurobiology of focal TLE (e.g., TLE vs. HP organization, TLE post- vs. pre-surgical organization, seizure outcome, pathology, seizure subtype, and seizure onset zone lateralization). In this project, we sought to determine the regional and whole brain functional connectome and structural features that are shared (or distinct) across these "conditions". Methods: Sample was comprised of 54 anterior temporal lobectomy-treated focal TLE patients and matched 32 healthy participants (HP) with presurgical/baseline and 1 year postsurgical/follow-up multi-modality MRI data. We calculated 8 local or network resting-state fMRI measures, along with gray matter volume for 116 brain regions. We then tested (independent/paired t-tests, FDR corrected) for the differential effects of these measures on key binary characteristics of the 6 TLE conditions.

Results: After t-test analysis of the 54 sets of statistics, we found that across "conditions" the thalamus played a prominent role in TLE. The ipsilateral thalamus was the brain region with the largest absolute mean Hedge's g effect size and displayed the largest number of "condition" discriminations (number significant at $p<0.05$, uncorrected). In terms of specific brain functional organization measures, thalamic local functional harmony (ReHo) characterized pre-surgical organization. Thalamic spontaneous activity (ALFF) and gray matter volume characterized post-surgical organization. Particularly noteworthy was increased ipsilateral thalamic ALFF, but decreased contralateral thalamic ALFF, postoperatively. Hubness (degree centrality), control (modal controllability) and ALFF were reliable predictors of poor seizure outcome (AUC = 0.79 in XGboost classifier, five-fold validation). Conclusions: The data clearly showed that each functional/structural feature plays a different role in each clinical context ("condition"), but in terms of regions, beyond even the temporal lobe, the thalamus has most consistent impact across "conditions".

Research In-Depth Presentations

CONNELLY AUDITORIUM, HAMILTON | 2:00PM — 3:30PM

Moderator: Arijita Ghosh, PhD

2:00 - 2:05	Lisa Kozlowski	Opening Remarks
2:05 - 2:25	Marilen Federico	Loss of DRP1 contributes to cardiac remodeling that promotes alterations in calcium handling and heart function leading to progressive heart failure
2:25 - 2:45	Melissa Molho	Defining the Role of Powassan Virus in Evading Host Antiviral Innate Immunity
2:45 - 3:05	Dipon Kumar Mondal	Decorin suppresses tumor lymphangiogenesis: A mechanism to curtail cancer progression
3:05 - 3:25	Ziaur Rahman	Ablation of Platelet Apoptosis Signal-Regulating Kinase 1 (Ask1) Protects Mice from Ischemic Stroke-Induced Remote Organ Injury (ROI)
3:25 - 3:30	Arijita Ghosh	Closing Remarks

09 Loss of DRP1 contributes to cardiac remodeling that promotes alterations in calcium handling and heart function leading to progressive heart failure

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Mitochondrial health is critical for ATP production sustaining cardiomyocyte excitation-contraction coupling (ECC). Dynamin-related protein 1 (DRP1), is a GTPase that participates in mitochondrial fission. Although DRP1 is widely expressed in the cytosol of cardiomyocytes, there is no clear physiological role described for it. We then questioned what is so critical in DRP1 function making the lack of the protein so harmful to the heart. We used αMHC-Mer-Cre-Mer (MCM) as control mice, and DRP1fl/fl to induce DRP1 KO (icKO) with three consecutive tamoxifen injections (40mg/Kg/day). We found that icKO mice live only 9 weeks after the induction demonstrating the lack of DRP1 in adult mice is fatal. Echocardiographic analysis showed that heart function falls over time (EF: from 67.40±3.3 to 28.08±7.5 and FS: from 34.10±4.3 to 13.18±5.1) together with an increment in premature ventricular beats appearance in icKO compared to MCM. To investigate this strong phenotype, we studied the proteome in icKO vs MCM. We found increased fold change in proteins related to cardiomyopathy, inflammation, Ca2+ handling, and mitochondrial function, and actin-cytoskeleton modulation. To determine whether this remodeling could affect heart function we studied cell shortening and cytosolic Ca2+. We found decreased cell shortening (0.09±0.01 vs 0.16±0.02) and increased cytosolic calcium (0.54±0.06 vs 0.31±0.03) together with spontaneous calcium release events (39.4±4.9 vs 17.9±8.2) in icKO demonstrating a reduced sensitivity in the myofilaments. By western blot, there was a depression of the myosin light chain with an increment in α-actinin that can make the sarcomere less sensitive and more rigid. We also investigated mitochondria morphology and functionality and found a depression in all supercomplexes activity, mitochondrial membrane potential (~50% reduction), and oxygen consumption (1.41±0.2 vs 3.27±0.4). Lack of DRP1 not only affects mitochondrial bioenergetics but also triggers myofilament changes that affect cardiomyocyte contraction leading to heart failure and arrhythmias.

19 Defining the Role of Powassan Virus in Evading Host Antiviral Innate Immunity

Molho M[1], O'Mara M[1], Blanco-Melo D[2], Johnson J[3], Ramage H[1]

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Flaviviruses are positive-strand RNA viruses comprised of several human pathogens accounting for significant morbidity and mortality worldwide. Powassan virus (POWV) is an emerging, neurotropic tick-borne flavivirus endemic in North America. Flaviviruses are highly sensitive to host innate immunity, including Type I interferon (IFN) signaling, and have evolved to antagonize these responses. However, these strategies have not been defined for POWV. We aim to identify the mechanisms by which POWV evades innate host responses. We used transcriptomics and proteomics in HMC3 microglia cells to define the host response to POWV infection. Comparison of our RNA-seq and proteome data revealed an increase in RNA of the IFN-stimulated gene (ISG) TRIM5 alpha but a decrease in TRIM5 alpha protein abundance, suggesting that POWV may target TRIM5 alpha protein for degradation. In follow-up studies, we confirm a decrease in TRIM5 alpha protein abundance during POWV infection by western blotting and show this is conserved in other tick-borne flaviviruses. We also demonstrate that TRIM5 alpha is antiviral in POWV infection using siRNA-mediated depletion and protein overexpression. To identify additional mechanisms of innate immune evasion by POWV, we used a luciferase reporter assay and found that the POWV NS5 protein inhibits the expression of ISGs following stimulation with Type I IFN. Our interactome studies revealed that POWV NS5 interacts with the Type I IFN signaling protein TYK2. Further, expression of POWV NS5 inhibits TYK2 phosphorylation and downstream Type I IFN signaling, suggesting a novel mechanism of antagonism. Together, our data demonstrate that POWV targets multiple components of the Type I IFN signaling pathway to subvert the host antiviral response. Ongoing studies are focused on understanding the viral and host components mediating TRIM5 alpha degradation and the mechanism by which POWV NS5 antagonizes TYK2. Together, these studies will shed light on POWV pathogenesis and host innate immune signaling and may inform future avenues for therapeutic development.

20 Decorin suppresses tumor lymphangiogenesis: A mechanism to curtail cancer progression

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The complex interaction between malignant cells and the cellular and molecular components of the tumor microenvironment is a key aspect of cancer growth and development. Soluble bioactive molecules such as proteoglycans often affect the crosstalk between these tumors and the host niche. Decorin, a small leucine-rich proteoglycan (SLRP) primarily expressed by stromal cells, exhibits onco-suppressive effects by interacting with several receptor tyrosine kinases (RTKs). Overall, decorin leads to the inhibition of RTK activity and attenuates the pro-angiogenic program of tumor cell proliferation. The query of the Cancer Genome Atlas (TCGA) reveals a significant reduction of decorin expression in solid tumors, providing the impetus to perform an unbiased transcriptomic analysis using deep RNAseq in breast carcinoma allografts treated with or without decorin. We found

that systemic delivery of decorin downregulates a cluster of tumor-associated genes involved in lymphatic vessel (LV) development, including Lyve1 and Podoplanin. We established that Lyve1 and Podoplanin, two signature markers of LVs, were markedly suppressed at both the mRNA and protein levels and this suppression correlated with a significant reduction in tumor LVs. Through an LV sprouting assay in an ex vivo 3D collagen model, we identified that suppression of lymphangiogenesis is a function unique to decorin as its proteoglycan homolog, biglycan did not affect LV sprouting. Furthermore, we found that decorin evoked autophagic degradation of Lyve1 in a nutrient- and energy-independent manner. Mechanistically it interacts with vascular endothelial growth factor receptor 3 (VEGFR3), the main lymphatic RTK, and its activity was required for the decorin-mediated block of lymphangiogenesis. Overall, these findings implicate decorin as one of the few biological factors capable of producing antilymphangiogenic activity, thereby providing evidence for a critical therapeutic agent for curtailing breast cancer metastasis.

24 Ablation of Platelet Apoptosis Signal-Regulating Kinase 1 (Ask1) Protects Mice from Ischemic Stroke-Induced Remote Organ Injury (ROI)

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Background: Platelets play an important role in CVD. ASK1 plays a critical role in platelet activation. Ischemic stroke-induced neuronal injury releases DAMPs and ROS that are known to activate platelets. We hypothesize that platelet activation after ischemic stroke may be responsible for ROI and increased mortality seen in stroke patients.

Aim: To evaluate the role of platelet Ask1 in aggravating ischemic stroke-induced ROI using transient middle cerebral artery occlusion, a murine stroke model. Methods: Platelet-specific Ask1 knock-out (Ask1fl/fl/Pf4Cre+) mice in a C57BL/6 background and littermate controls (Ask1fl/fl) were used. Ischemic stroke was induced by 60 minutes of ischemia followed by 72 hours of reperfusion. The activated platelets were assessed by PE-conjugated Jon-A binding. Miles assay was used to evaluate vascular and gut permeability. Brain infarct was measured by TTC staining of the brain slices. H&E staining was used to assess the intestinal injury. Results: Ask1fl/fl/Pf4Cre+ mice had a stroke-induced neurological impairment of 2-3 on an NIH stroke scale of 0-4, comparable to that seen in Ask1fl/fl mice (n=5). The brain infarct volume was also similar in both strains (n=5), suggesting that ablation of platelet Ask1 does not affect the extent of brain injury. Interestingly, after stroke, Ask1fl/fl mice showed more Jon-A-positive platelets than Ask1fl/fl/Pf4Cre+ mice, indicating that as a result of stroke, platelets are activated and lack of Ask1 resist this activation. H&E staining of Ask1fl/fl mice ileum showed villous atrophy which was reduced in Ask1fl/fl/Pf4Cre+ mice, indicating protection from intestinal injury after stroke. Additionally, Ask1fl/fl/Pf4Cre+ mice showed significantly reduced gut permeability after stroke compared to Ask1fl/fl mice (n=5). Furthermore, lung vascular permeability was also attenuated in Ask1fl/fl/Pf4Cre+ mice compared to Ask1fl/fl mice. Conclusion: Platelets lacking Ask1 resist activation and reduce ROI upon ischemic stroke. Thus, the inhibition of platelet Ask1 may reduce post-stroke ROI and mortality.

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