

UNIVERSITY OF PITTSBURGH

NINETEENTH

*POSTDOCTORAL
RESEARCH
SYMPOSIUM*

April 29, 2026 | 1-5pm
William Pitt Union

April 29, 2026

Dear Colleagues,

Welcome to the 19th Annual University of Pittsburgh Postdoctoral Research Symposium! This event is dedicated to the University's postdoctoral professionals – a highly talented and innovative group of early-stage investigators whose passion and commitment to research fuels new discoveries every day. We are thrilled and honored for you to join us as we recognize and share the impactful work being conducted by our community.

This symposium celebrates these outstanding contributions while providing a valuable opportunity to exchange ideas, build collaborations, and simply connect with one another across disciplines. We are excited to bring back our Networking Bingo game and People's Choice Award voting. We are also incorporating "table topics" during the networking session to facilitate your discussions this year. We encourage you to engage with your peers, explore the diverse research on display, and take advantage of this unique space.

We are proud to continue our tradition of presenting professional development awards to outstanding poster presentations as determined by our judges and attendees. These awards are made possible by the sustained and generous support of our University community. This year, we will recognize 15 postdoctoral professionals through 10 Best Poster Awards and 5 People's Choice Awards.

This symposium would not be possible without the support from the Office of Academic Career Development, the Office of the Senior Vice Chancellor for the Health Sciences, the Office of the Provost, our University sponsors, and NovogeneUSA. We also thank our poster judges and volunteers for their essential role in making today's event a success, and we extend our sincere gratitude to Dr. Rob Rutenbar, Dr. Anantha Shekhar, and Dr. Amanda Godley for joining us and for their commitment to the postdoctoral community.

Finally, thank you to postdocs, faculty, and other members of the Pitt community for being part of this special day. We hope you find inspiration, connections, and opportunities to further your research journey. Enjoy the symposium!

---- 2026 UPPDA Executive Board

President: Jessica Nuwer, PhD

Advocacy Chair: Jonathan Perdomo, PhD

Career Development Chair: Afsana Naaz, PhD

Communications Chair: Mary Cacace, PhD

Diversity and Inclusion Chair: Rain Katz, PhD

International Chair: Monojit Bhattacharjee, PhD

Networking Chair: Marie-Charlotte Allichon, PhD

Executive Board Officers 2025-2026

President	Jessica Nuwer, PhD School of Medicine Department of Pharmacology & Chemical Biology j.nuwer@pitt.edu
Advocacy Committee Chair	Jonathan Perdomo, PhD School of Medicine Department of Biomedical Informatics jep386@pitt.edu
Career Development Chair	Afsana Naaz, PhD School of Engineering Department of Chemical Engineering afn14@pitt.edu
Communication Chair	Mary Cacace, PhD School of Medicine Department of Ob/Gyn & Reproductive Sciences mac519@pitt.edu
Diversity and Inclusion Chair	Rain Katz, PhD School of Medicine Department of Neurology rain.katz@pitt.edu
International Committee Chair	Monojit Bhattacharjee, PhD School of Medicine Department of Dermatology mob114@pitt.edu
Networking Chair	Marie-Charlotte Allichon, PhD School of Medicine Department of Psychiatry maa1128@pitt.edu

UPPDA Volunteers 2025-2026

ADVOCACY COMMITTEE

Samuel Johnson, PhD	Department of Pathology, School of Medicine
Ashley Shafer, PhD	Office of Child Development, School of Education
Sam Nunez, PhD	Department of Behavioral & Community Health Sciences, School of Public Health
Casey Becker, PhD	Department of Neurosurgery, School of Medicine
Kaushik Sreeram, MBBS	Department of Rheumatology, School of Medicine

CAREER DEVELOPMENT COMMITTEE

Janet Bih, PhD	Learning Research & Development Center
Ipsita Dey, PhD	Department of Pharmacology & Chemical Biology, School of Medicine
Andzoa Jamus, PhD	Department of Medicine, School of Medicine
Ashish Dhayani, PhD	Department of Dermatology, School of Medicine
Joyce Li, PhD	Department of Civil & Environmental Engineering, Swanson School of Engineering

COMMUNICATIONS COMMITTEE

Mary Cundiff, PhD	Department of Immunology, School of Medicine
Mary Melissa Roland, PhD	Department of Immunology, School of Medicine

INTERNATIONAL COMMITTEE

Julia Chagas, PhD	Department of Orthopaedic Surgery, School of Medicine
Rupali Mittal, PhD	Department of Chemical & Petroleum Engineering, Swanson School of Engineering
Samira Raminfard, PhD	Department of Psychiatry, School of Medicine
Rafaela José da Silva, PhD	Department of Biological Sciences, Dietrich School of Arts & Sciences
Akanxa Rooney, PhD	Department of Pediatrics, School of Medicine
Arundhati Gupta, PhD	Department of Microbiology & Molecular Genetics, School of Medicine
Daphne Rodriguez, PhD	Department of Immunology, School of Medicine
Kamakshi Mehta, PhD	Department of Ophthalmology, School of Medicine
Aswathy BS, PhD	Department of Psychiatry, School of Medicine

NETWORKING COMMITTEE

Julia Chagas, PhD	Department of Orthopaedic Surgery, School of Medicine
Anumeha Saha, PhD	Department of Civil & Environmental Engineering, Swanson School of Engineering
Zeynep Erdogan-Yildirim, PhD	Department of Oral & Craniofacial Sciences, School of Dental Medicine
Carolina Soares, PhD	Department of Psychiatry, School of Medicine
Kamakshi Mehta, PhD	Department of Ophthalmology, School of Medicine
Manohar Koduri, PhD	Department of Cardiothoracic Surgery, School of Medicine

COMMUNITY AMBASSADORS

Anna Korol, PhD	Department of Radiology, School of Medicine
Sagar Dhoble, PhD	Department of Chemical & Petroleum Engineering, Swanson School of Engineering
Syamprasad NP, PhD	Center for Pharmacogenetics, School of Pharmacy
Vik Meadows, PhD	Department of Pharmacology & Chemical Biology, School of Medicine
Ashish Dhayani, PhD	Department of Dermatology, School of Medicine
Adam Officer, PhD	Hillman Cancer Center, School of Medicine

AGENDA
April 29, 2026 | 1-5 PM
WILLIAM PITT UNION

10:00 AM-12:30 PM	Poster Set-Up & Check-in <i>Lobby</i>
12:00 – 1:00 PM	Judge and Guest Check-In <i>Lobby</i>
1:00-2:00 PM	Poster Session 1 Odd Numbered Posters <i>Ballroom, Kurtzman Room, and Assembly Room</i>
2:00-3:00 PM	Poster Session 2 Even Numbered Posters <i>Ballroom, Kurtzman Room, and Assembly Room</i>
3:00-4:00 PM	Networking Reception Refreshments and “Table Topics” <i>William Pitt Union Lower Lounge</i>
4:00-5:00 PM	Awards Ceremony <i>William Pitt Union Lower Lounge</i>

POSTDOCTORAL LEADERSHIP AWARD WINNER



Dr. Ashley E. Shafer

Ashley E. Shafer, PhD (she/her), is a scholar-practitioner and early childhood advocate recognized for her leadership in advancing systems change, community engagement, and advocacy within and beyond the University of Pittsburgh. At the University of Pittsburgh's Office of Child Development, she leads research-to-practice initiatives and forges university-community partnerships to enhance services for children and families. Her work integrates rigorous evaluation, implementation science, and community collaboration to translate evidence into practical tools and policies across early childhood programs.

Trained in Applied Developmental Psychology with a minor in Research Methodology, Dr. Shafer bridges research and practice by translating evidence into actionable strategies that support children, families, and the early childhood workforce. Her areas of focus include early relational health, workforce well-being, family engagement, and culturally responsive practices. She has led initiatives in relationship-based professional learning, program evaluation, and data-informed decision-making, with a strong commitment to advancing equity in under-resourced communities.

In addition to her programmatic work, Dr. Shafer has made significant contributions to the postdoctoral community. She served as President of the University of Pittsburgh Postdoctoral Association (2024-2025), where she strengthened organizational infrastructure, advocated for postdoctoral independence, and elevated postdoc voices across the institution. She has also represented postdocs at the national level through her involvement with the National Postdoctoral Association Advocacy Committee and contributed to university governance through the Senate Government Relations Committee. Her leadership has focused on improving systems, communication, and institutional support for all postdocs. Outside of her professional work, Dr. Shafer enjoys spending time with her husband, Joe, and their cat, McGregor, and deeply values her role as an aunty and the time she spends with her chosen family. She also enjoys cheering on Pittsburgh sports teams, traveling, and serving her local community through volunteer work with the Junior League of Pittsburgh.

ABOUT THE POSTDOCTORAL LEADERSHIP AWARD This award, formerly known as the Alumni Award, honors a postdoctoral professional who has demonstrated profound and visionary leadership within the UPPDA. The awardee is recognized for their high-impact initiatives and strategic contributions that have significantly shaped the UPPDA trajectory. Candidates are nominated and selected by the UPPDA Executive Board.

Past Alumni Award Recipients

2025 – Rachael Rush, PhD	2014 – Catherine Haggerty, PhD
2024 – Mohammadreza Tabary, MD	2012 – Aaron Bell, PhD
2023 – David Gau, PhD	2011 – Natacha De Genna, PhD
2019 – Kimberly Payne, PhD	2010 – Lei Hong, PhD
2018 – Chelsea Stillman, PhD	2009 – David Robinson, PhD
Collin Diedrich, PhD	2008 – Richard Bodnar, PhD
2017 – Karen Carney, PhD	2007 – Steven Wendell, PhD
2016 – John Merriam, PhD	2006 – Elsa Strotmeyer, PhD
2014 – Catherine Haggerty, PhD	2005 – Stuart Olmsted, PhD
2013 – Timothy Maul, PhD	

INAUGURAL POSTDOCTORAL SERVICE AWARD WINNER



Dr. Anna S. Korol

Anna S. Korol, PhD, is a Postdoctoral Associate in the Department of Radiology at the University of Pittsburgh. She received her BS in micro- and nanoelectronics in 2020 from Igor Sikorsky Kyiv Polytechnic Institute in Ukraine and PhD in neuroscience in 2025 from West Virginia University. Her graduate research focused on developing innovative quantification methods and biomimetic assistive technologies aimed at improving rehabilitation and the quality of life for individuals recovering from neuromuscular injuries, such as stroke or peripheral nerve damage. Her work was recognized with honors, including serving as Associate Scholar for Stroke, and Its Alzheimer's Disease Related Dementias (ADRD) T32, 2-

year Trainee Pilot Grant from the Center for Foundational Neuroscience Research and Education at West Virginia University, and Trainee Professional Development Award from Society for Neuroscience.

Currently, her work in Dr. Takashi (TK) Kozai's lab investigates the single-cell neural responses to intracortical microstimulation in the visual and somatosensory cortices with an overarching goal to design robust and effective neurotechnology for individuals with neural injuries. Beyond the lab, Dr. Korol is passionate about fostering a vibrant postdoctoral community and promoting best mentorship practices for undergraduate and graduate students. She currently serves as a Community Ambassador for the University of Pittsburgh Postdoctoral Association (UPPDA) and on the Postdoctoral Committee of the Center for the Neural Basis of Cognition (CNBC).

ABOUT THE POSTDOCTORAL SERVICE AWARD This award honors a postdoctoral professional who has provided outstanding service to the UPPDA through sustained and tireless commitment. The awardee is recognized for their reliability and proactive participation in UPPDA initiatives that ensure the success of postdoctoral programming. Candidates are nominated and selected by the UPPDA Executive Board.

POSTDOCTORAL ADVOCATE AWARD WINNER



Meghan S. Culpepper

Meghan Culpepper, MS, joined the Office of the Provost at the University of Pittsburgh in 2017 and has served as the *Postdoctoral Affairs and Graduate Fellowships Manager* since 2024. In this role she supports postdoctoral researchers and graduate students through program coordination, project management, and strategic communication. Her work centers on building efficient processes and supportive infrastructures so graduate students and postdocs can thrive in their research, training, and career development. She works collaboratively with the University of Pittsburgh Postdoctoral Association (UPPDA), the Center for Postdoctoral Affairs in the Health Sciences, and with schools and programs to develop and strengthen the

resources and support Pitt provides to the postdoc community. Meghan's path to postdoctoral advocacy is grounded in a strong scientific and educational foundation. She holds an MS in Environmental Science, Policy & Management from the University of California, Berkeley, and an MS in Biology (Ecology, Evolution & Conservation Biology) and BS in Biology (Zoology) from San Francisco State University. This background has informed her longstanding commitment to mentorship, support, and fostering inclusive academic communities.

ABOUT THE POSTDOCTORAL ADVOCATE AWARD This award honors an individual who has demonstrated exceptional commitment to improving the postdoctoral experience at Pitt. The awardee is recognized for their advocacy efforts on policies that benefit the postdoctoral community and for promoting equity, diversity, and inclusion. Candidates are nominated and selected by the UPPDA Executive Board.

Past Advocate Award Recipients

2025 – Laura Miller, PhD, PC	2013 – Charles Nieman, PhD, DSO, ARO
2024 – Ian Sigal, PhD	2012 – Darlene Zellers, PhD
2023 – Amanda Godley, PhD	2011 – Christopher Martin, PhD
2019 – Nathan Urban, PhD	2010 – Patricia Beeson, PhD
2018 – Thomas Smithgall, PhD	2009 – Bruce Freeman, PhD
2017 – Arvind Suresh, MS	2008 – Andrew Blair, PhD
2016 – Jennifer Woodward, PhD	2007 – Joan Lakoski, PhD
2015 – Ora Weisz, PhD	2006 – Elizabeth Baranger, PhD
2014 – Alan Sved, PhD	2005 – Arthur Levine, MD

POSTDOCTORAL MENTOR AWARD WINNER



Dr. Kristine A. Wilckens

Dr. Kristine Wilckens is an Associate Professor of Psychiatry in the Center for Sleep and Circadian Science. Her research focuses on the role of sleep in promoting brain health and cognitive fitness. Her methodological expertise includes interventions that enhance aspects of sleep that are most restorative to brain health, including behavioral and neuromodulatory interventions.

Dr. Wilckens obtained graduate training in Cognitive Psychology and Cognitive Neuroscience at New York University and the University of Pittsburgh Department of Psychology where she obtained her PhD in 2012. Following a postdoctoral fellowship in the Department of Psychiatry at the University of Pittsburgh, she was awarded a K01 Career

Development award through which she gained training in neuroimaging, advanced sleep methods, and neuropsychological assessment. She was appointed to Assistant Professor in the Department of Psychiatry in October 2015 and Associate Professor in June 2024.

Dr. Wilckens is passionate about mentoring early career scientists in identifying and developing their unique research programs and advocating for their career development. She serves as a faculty mentor for the Translational Sleep and Circadian Research T32 program (Director: Buysse), the Bioengineering in Psychiatry Graduate Training Program (Directors: Aizenstein, Ibrahim), and the Departments of Psychiatry and Psychology. She is chair of the Alzheimer's Association Professional Interest Group on Sleep and Circadian Rhythms which provides networking and educational opportunities for investigators at the international level. She is a member of the graduate faculty and leads formal workshops in mentor training through the University of Pittsburgh Center for Mentoring. She has served on academic committees for students, postdocs, and faculty in many departments, including Psychiatry, Psychology, Neuroscience, Nursing, Communication Disorders, Epidemiology as well as outside the University. She has chaired several professional development workshops and research symposia aimed educating the next generation of investigators in the field of sleep and circadian science.

ABOUT THE POSTDOCTORAL MENTOR AWARD Modeled after the National Postdoctoral Association (NPA) Mentor Award, this award honors a faculty member who engages in exceptional mentoring of postdoctoral professionals. The awardee is recognized for their ability to foster professional growth and career readiness. Candidates are nominated by the postdoctoral community and selected by the UPPDA Executive Board.

Past Mentor Award Recipients

2025 – Michael S. Gold, PhD

2024 – Michele D. Levine, PhD

2023 – Ian Scott, PhD

2019 – Ronald Buckanovich, PhD

2018 – Tina Goldstein, PhD

2017 – Brooke Molina, PhD

2016 – Melissa Bilec, PhD

2015 – Jennifer Silk, PhD

POSTER PRESENTATION & AWARD GUIDELINES

Posters will be judged from 1:00 PM to 3:00 PM.

Presenters with **odd** numbered posters will be judged from 1:00 PM to 2:00 PM.

Presenters with **even** numbered posters will be judged from 2:00 PM to 3:00 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, while the People's Choice presenters will receive a \$100 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 2026 and May 2027. The award categories include:

- *Basic Biomedical and Pharmacological Sciences*
- *Behavioral Sciences and Humanities, and Education and Curriculum*
- *Cellular and Molecular Biology*
- *Clinical and Surgical Sciences*
- *Engineering and Physical, Natural, 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 ceremony following the networking session.

POSTER PRESENTATION AWARD SPONSORS

GOLD SPONSORS

\$501 - \$1,000

Learning Research & Development Center

Department of Radiology
School of Medicine

SILVER SPONSORS

\$251 - \$500

Department of Cell Biology
School of Medicine

Department of Computational and Systems Biology
School of Medicine

Department of Neurobiology
School of Medicine

Department of Pediatrics
School of Medicine

Department of Psychiatry
School of Medicine

Department of Surgery
School of Medicine

Bronze SPONSORS

\$1 - \$250

Vascular Medicine Institute
School of Medicine

INDEX OF POSTER PRESENTERS

Abstracts are in alphabetical order starting on page 17.

Poster	Name	Category	Email
1	Abbasi, Naghmeh	Basic Biomedical and Pharmacological Sciences	naa348@pitt.edu
2	Acharya, Vibha	Basic Biomedical and Pharmacological Sciences	via16@pitt.edu
3	Adamczyk, Natalie	Basic Biomedical and Pharmacological Sciences	NAA464@pitt.edu
83	Ajam, Ali	Clinical and Surgical Sciences	ala449@pitt.edu
55	Arrigo, Austin	Cellular and Molecular Biology	arrigoa@upmc.edu
4	Atale, Neha	Basic Biomedical and Pharmacological Sciences	NEA48@pitt.edu
43	Avni, Inbar	Behavioral Sciences and Humanities, Education and Curriculum	avi11@pitt.edu
56	Bajaj, Anjali	Cellular and Molecular Biology	anb705@pitt.edu
5	Balasubramanian, Hari Deva Muthu	Basic Biomedical and Pharmacological Sciences	hab273@pitt.edu
84	Balogun, Wasiu	Clinical and Surgical Sciences	wgb19@pitt.edu
57	Bommana, Raghunath Reddy	Cellular and Molecular Biology	rrb71@pitt.edu
85	Borkowski, Vera	Clinical and Surgical Sciences	vmb56@pitt.edu
6	Brockway, Dakota	Basic Biomedical and Pharmacological Sciences	dab550@pitt.edu
7	Bugga, Paramesha	Basic Biomedical and Pharmacological Sciences	buggap@pitt.edu
103	Campos, Vinicius	Engineering and Physical, Natural, and Computational Sciences	vpc24@pitt.edu
58	Cetinkaya Hosafci, Gamze	Cellular and Molecular Biology	gtc31@pitt.edu
104	Chandrashekhar, Raghuveer	Engineering and Physical, Natural, and Computational Sciences	rac502@pitt.edu
8	Chen, Shangyu	Basic Biomedical and Pharmacological Sciences	shc315@pitt.edu
9	Cheng, An-Chi	Basic Biomedical and Pharmacological Sciences	anc907@pitt.edu
59	Cooper, Anthonya	Cellular and Molecular Biology	anc422@pitt.edu
10	Correll, Erika	Basic Biomedical and Pharmacological Sciences	eac197@pitt.edu
11	Cuevas, Rolando	Basic Biomedical and Pharmacological Sciences	rac218@pitt.edu
60	Cundiff, Mary	Cellular and Molecular Biology	mdc179@pitt.edu
118	Dahmani, Larbi Zakaria	Engineering and Physical, Natural, and Computational Sciences	zakaria@pitt.edu

Poster	Name	Category	Email
86	de Rosa, Cristina	Clinical and Surgical Sciences	c.b.derosa@pitt.edu
61	Deshpande, Rushikesh	Cellular and Molecular Biology	rhd10@pitt.edu
62	Dev, Gagan	Cellular and Molecular Biology	GAD103@pitt.edu
44	Dickens, Jacinta	Behavioral Sciences and Humanities, Education and Curriculum	jad710@pitt.edu
105	Dieckhaus, Laurel	Engineering and Physical, Natural, and Computational Sciences	LAD311@pitt.edu
12	Divens, Ashley	Basic Biomedical and Pharmacological Sciences	amd669@pitt.edu
106	Edakalavan, Smitha	Engineering, Physical, and Computational Sciences	sme57@pitt.edu
13	Elhaw, Amal	Basic Biomedical and Pharmacological Sciences	ate29@pitt.edu
14	Erdogan-Yildirim, Zeynep	Basic Biomedical and Pharmacological Sciences	zee5@pitt.edu
87	Falbo, Kierra	Clinical and Surgical Sciences	kjf111@pitt.edu
45	Feldman, Julia	Behavioral Sciences and Humanities, Education and Curriculum	feldmanjs2@upmc.edu
107	Fetters, Andrea	Engineering and Physical, Natural, and Computational Sciences	amf436@pitt.edu
15	Galgano, Luca	Basic Biomedical and Pharmacological Sciences	lucag@pitt.edu
108	Ganjee, Razieh	Engineering and Physical, Natural, and Computational Sciences	r_ganjee@pitt.edu
46	Gray, Cecily A	Behavioral Sciences and Humanities, Education and Curriculum	cag414@pitt.edu
110	Gretok, Evan	Engineering and Physical, Natural, and Computational Sciences	ewg13@pitt.edu
63	Habli, Zeina	Cellular and Molecular Biology	zeh37@pitt.edu
64	Hong, Veronica	Cellular and Molecular Biology	veh34@pitt.edu
16	Jain, Siddhi	Basic Biomedical and Pharmacological Sciences	sij55@pitt.edu
17	Jamus, Andzoa	Basic Biomedical and Pharmacological Sciences	ANJ321@pitt.edu
18	Johnson, Samuel	Basic Biomedical and Pharmacological Sciences	sdj48@pitt.edu
19	Joy, Jincy	Basic Biomedical and Pharmacological Sciences	jij107@pitt.edu
47	Jusko, Morgan	Behavioral Sciences and Humanities, Education and Curriculum	juskoml@upmc.edu
20	Kamath, Manali	Basic Biomedical and Pharmacological Sciences	mak996@pitt.edu
21	Kozel, Jenesis	Basic Biomedical and Pharmacological Sciences	jdg110@pitt.edu

Poster	Name	Category	Email
111	Kumar, Anal	Engineering and Physical, Natural, and Computational Sciences	ank741@pitt.edu
22	Kumar, Rahul	Basic Biomedical and Pharmacological Sciences	rahul.kumar@pitt.edu
109	Kumar, Ravinder	Engineering and Physical, Natural, and Computational Sciences	rak324@pitt.edu
88	Lee, You-Rim	Clinical and Surgical Sciences	you-rim.lee@pitt.edu
23	Li, Yuzhen	Basic Biomedical and Pharmacological Sciences	liy38@upmc.edu
112	Liu, Yen-Tung	Engineering and Physical, Natural, and Computational Sciences	trl117@pitt.edu
65	Mabry, Samuel	Cellular and Molecular Biology	sjm255@pitt.edu
66	Maestas, Jr., David	Cellular and Molecular Biology	DRM154@pitt.edu
24	Marin, Paula	Basic Biomedical and Pharmacological Sciences	paula.marin@pitt.edu
67	Maurya, Sudeep Kumar	Cellular and Molecular Biology	skm64@pitt.edu
68	Mazumder, Md Habibul Hasan	Cellular and Molecular Biology	mdm238@pitt.edu
25	Melcher, Amber	Basic Biomedical and Pharmacological Sciences	AVM108@pitt.edu
89	Mroue, Rayan	Clinical and Surgical Sciences	ram518@pitt.edu
26	Mueller Brown, Karina	Basic Biomedical and Pharmacological Sciences	kmb520@pitt.edu
27	Munyoki, Sarah	Basic Biomedical and Pharmacological Sciences	skm53@pitt.edu
28	Nagpal, Ashna	Basic Biomedical and Pharmacological Sciences	asn68@pitt.edu
29	Nwagbo, Uzoamaka	Basic Biomedical and Pharmacological Sciences	uan7@pitt.edu
69	Osman, Guled	Cellular and Molecular Biology	GAO63@pitt.edu
70	Ozdemir, Isa	Cellular and Molecular Biology	zd1@pitt.edu
90	Pant, Arjun	Clinical and Surgical Sciences	arp466@pitt.edu
91	Pant, Arjun	Clinical and Surgical Sciences	arp466@pitt.edu
113	Park, Sangmi	Engineering and Physical, Natural, and Computational Sciences	sap545@pitt.edu
48	Park, Yul Min	Behavioral Sciences and Humanities, Education and Curriculum	yup58@pitt.edu
72	Patel, Priyal	Cellular and Molecular Biology	prp179@pitt.edu
71	Patel, Yash	Cellular and Molecular Biology	yap22@pitt.edu

Poster	Name	Category	Email
49	Pham, Holly	Behavioral Sciences and Humanities, Education and Curriculum	phamht2@upmc.edu
50	Phillips, Meredith	Behavioral Sciences and Humanities, Education and Curriculum	mlp172@pitt.edu
73	Pinero Robles, Carlos	Cellular and Molecular Biology	CAP215@pitt.edu
30	Pinto, Alejandro	Basic Biomedical and Pharmacological Sciences	AVE21@pitt.edu
92	Poala, Guilherme	Clinical and Surgical Sciences	gup11@pitt.edu
51	Prakash, Krithika	Behavioral Sciences and Humanities, Education and Curriculum	prakashk@upmc.edu
74	Rasheed Issa, Dalia	Cellular and Molecular Biology	dar258@pitt.edu
93	Rawls, Bobby	Clinical and Surgical Sciences	bgr33@pitt.edu
31	Reddy, Aravind	Basic Biomedical and Pharmacological Sciences	aravindr@pitt.edu
94	Redinger, Allen	Clinical and Surgical Sciences	allen.redinger@pitt.edu
52	Rienne, Gavin	Behavioral Sciences and Humanities, Education and Curriculum	gir71@pitt.edu
32	Rizwan, Saira	Basic Biomedical and Pharmacological Sciences	sar705@pitt.edu
75	Robert, Nadine	Cellular and Molecular Biology	nar102@pitt.edu
95	Rocha, Andreia	Clinical and Surgical Sciences	anr436@pitt.edu
76	Rossillo, Mary	Cellular and Molecular Biology	rossillo@pitt.edu
33	Rozovsky, Renata	Basic Biomedical and Pharmacological Sciences	rer92@pitt.edu
96	Ruppert, Emma	Clinical and Surgical Sciences	emr269@pitt.edu
34	Rush, Rachael	Basic Biomedical and Pharmacological Sciences	r.rush@pitt.edu
35	Sanotra, Monika	Basic Biomedical and Pharmacological Sciences	mol81@pitt.edu
36	Satterwhite, Rebecca	Basic Biomedical and Pharmacological Sciences	satterwhite@pitt.edu
97	Scop Medeiros, Marina	Clinical and Surgical Sciences	mas1479@pitt.edu
98	Shi, Ruyu	Clinical and Surgical Sciences	rus39@pitt.edu
114	Shipp, James	Engineering and Physical, Natural, and Computational Sciences	james.shipp@pitt.edu
77	Shvedov, Naomi	Cellular and Molecular Biology	nrs163@pitt.edu
37	Singh, Sanpreet	Basic Biomedical and Pharmacological Sciences	sas1165@pitt.edu
53	Smith, Brittany	Behavioral Sciences and Humanities, Education and Curriculum	bts96@pitt.edu

Poster	Name	Category	Email
99	Soares, Carolina	Clinical and Surgical Sciences	soaresk@pitt.edu
115	Sung, Myung Kyun	Engineering and Physical, Natural, and Computational Sciences	mys46@pitt.edu
78	Thale, Insa	Cellular and Molecular Biology	ilt17@pitt.edu
54	Totoni, Samantha	Behavioral Sciences and Humanities, Education and Curriculum	SKC35@pitt.edu
79	Vaughn, Stephanie	Cellular and Molecular Biology	snv44@pitt.edu
116	Wang, Liyi	Engineering and Physical, Natural, and Computational Sciences	jow192@pitt.edu
38	Wherry, Jim	Basic Biomedical and Pharmacological Sciences	jdww194@pitt.edu
80	White, Sierra	Cellular and Molecular Biology	srw99@pitt.edu
100	Whitman, Jacob	Clinical and Surgical Sciences	jaw617@pitt.edu
101	Yang, Sheng	Clinical and Surgical Sciences	shy229@pitt.edu
39	Ye, Yansong	Basic Biomedical and Pharmacological Sciences	yay167@pitt.edu
40	Yilmaz, Sema Zeynep	Basic Biomedical and Pharmacological Sciences	yilmazs@pitt.edu
102	Yip, Catherine	Clinical and Surgical Sciences	cyy9@pitt.edu
42	Yu, Wenchao	Basic Biomedical and Pharmacological Sciences	WEY113@pitt.edu
81	Yu, Xiaojie	Cellular and Molecular Biology	xiaojie.yu@pitt.edu
117	Zhu, Jinli	Engineering and Physical, Natural, and Computational Sciences	jinli.zhu@pitt.edu
41	Zou, Han	Basic Biomedical and Pharmacological Sciences	hanzou@pitt.edu
82	Zou, Han	Cellular and Molecular Biology	hanzou@pitt.edu

3D-Printed Vascular Models Recapitulate Mechanical Heterogeneity and Guide VSMC Mechanosensitive Signaling in PAH

Abbasi, Naghmeh¹; Tai, Miranda¹; Chan, Stephen Y^{1,2}; Shiowski, Daniel^{1,2,3*}

¹Department of Medicine, University of Pittsburgh School of Medicine

²Pittsburgh Heart, Lung, and Blood Vascular Medicine Institute

³Department of Bioengineering, University of Pittsburgh

Background: Pulmonary arterial hypertension (PAH) is a progressive vascular disease characterized by vascular remodeling, and endothelial dysfunction, ultimately leading to death. Increasing evidence indicates that extracellular matrix (ECM) stiffening alters mechanotransduction and contributes to early disease progression. However, conventional in vitro models fail to replicate the mechanical heterogeneity of the vascular microenvironment. This study aimed to develop a biomimetic 3D-printed vascular scaffold with tunable stiffness and structural alignment to investigate mechanosensitive vascular smooth muscle cell (VSMC) responses relevant to PAH pathogenesis.

Methods: Three-dimensional vascular scaffolds were fabricated using GelMA and PEGDA hydrogels via stereolithography (BionovaX printer), incorporating aligned, non-aligned, and multimaterial configurations with and without grayscale photomask-defined stiffness gradients. Healthy and diseased mechanical environments were engineered by modulating scaffold composition and architecture. Nanomechanical properties were quantified using nanoindentation to map local compressive moduli, generating regions of high (28 kPa) and low (4 kPa) stiffness. Alignment was induced via microtopographical cues and verified through cytoskeletal staining. Human VSMCs were seeded onto scaffolds and cultured under controlled conditions. Mechanosensitive responses were evaluated by YAP1 nuclear localization and quantitative PCR of downstream genes.

Results: Alignment cues enhanced cytoskeletal organization, VSMC elongation, and directional orientation. Nanoindentation confirmed continuous stiffness gradients in photomask-patterned scaffolds, while unpatterned scaffolds showed homogeneous mechanics. Scaffold stiffness and architecture significantly regulated mechanosensitive responses. Multimaterial scaffolds without photomask exhibited the highest YAP1 nuclear translocation and greatest expression of YAP-regulated genes, particularly ANKRD1, followed by aligned scaffolds without photomask. Photomask-defined stiffness gradients showed lower gene expression compared to uniform stiff scaffolds, indicating that spatial mechanical heterogeneity modulates YAP-dependent transcriptional activation.

Conclusions: This biomimetic 3D-printed vascular model recapitulates key mechanical and structural features of PAH. Increased ECM stiffness and alignment promote YAP-mediated mechanotransduction and gene expression associated with vascular remodeling. This platform provides a powerful tool for studying PAH mechanobiology and therapeutic screening.

Genome-wide association analyses of cognitive domains in community- and clinic-based cohorts

Acharya, Vibha¹; Fan, Frank¹; Snitz, Beth²; Kamboh, M. Ilyas^{1,3}

¹Department of Human Genetics, University of Pittsburgh

²Department of Neurology, University of Pittsburgh

³Department of Psychiatry, University of Pittsburgh

Cognitive performance has a substantial genetic component. Cognitive performance across different neurocognitive domains varies with age. Identification of the genetic component of cognitive performance in older age can help to decipher underlying molecular pathways and thus devise novel therapeutic avenues. We analyzed harmonized cognitive scores of four domains (executive function, language, memory and visuospatial function) from 2605 subjects in a community-based Ginkgo Evaluation of Memory (GEM) cohort and 2057 clinically ascertained University of Pittsburgh Alzheimer's Disease Research Center cohort to assess genetic influences on baseline cognitive performance and longitudinal change. Genetic associations were tested using a generalized linear mixed-effects model with random intercepts and slopes, enabling joint inference of marginal genetic effects and SNP $\times$ age interactions.

The known genetic risk of *APOE4* was found to be significantly associated with all four neurocognitive domains in ADRC and with memory and executive function in GEM ($P_{\text{joint}}=3.14\text{E-}08$ to $8.23\text{E-}46$). The effect of *APOE* on all cognitive domains was attributed to its main effect rather than the interaction effect. In the ADRC cohort, we observed a novel association on chromosome 2 with the language domain ($P_{\text{joint}}=2.15\text{E-}08$), mainly due to its main effect ($P_{\text{marginal}}=3.42\text{E-}08$, $\beta=0.069$). We also observed significant variants in 129 genes (P range= $1.61\text{E-}11$ to $4.96\text{E-}08$) to be associated with memory in the GEM cohort where genetic effects were primarily attributed to their interaction effect with age. Of the 129 genes, only 2 were found to be significantly associated with memory in ADRC, indicating that clinical- and community-based cohorts could be modelling different genetic effects differing in aging phenotypes. While preliminary analysis suggested these genes are involved in cytoskeletal assembly, oxidative stress, and lipid processing, future analysis focusing on gene-pathway analysis will help to decipher the biological role of the associated genes.

Pain Prevalence and the Impact of Opioids in HPV+ Head and Neck Cancer

Adamczyk, Natalie¹; Mazul, Angela²; Nilsen, Marci^{2,3}; Atherton, Megan¹; Scheff, Nicole^{1,3}

¹Department of Neurobiology, School of Medicine, University of Pittsburgh

²Department of Otolaryngology, School of Medicine, University of Pittsburgh

³Hillman Cancer Center, University of Pittsburgh Medical Center

The incidence of HPV-positive head and neck cancer (HNC) is rapidly rising, with HPV-associated oropharyngeal cancer now surpassing cervical cancer as the most common HPV-related malignancy in the United States. While opioids are commonly used for cancer pain management, their immunomodulatory effects may influence oncologic outcomes. The incidence of patient-reported pain in HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) and the prognostic implications of opioid use at diagnosis are unknown.

We utilized two retrospective cohorts to compare patient-reported pain and perineural invasion (PNI) in HPV-positive and HPV-negative OPSCC. Patients were categorized by opioid use at diagnosis, and Kaplan-Meier survival curves estimated overall survival. Multivariable Cox proportional hazards regression evaluated associations between opioid use and survival, adjusting for T stage, nodal stage, age, and radiation therapy. We used an HPV-like HNC mouse model to recapitulate clinical disease, quantify orofacial nociceptive behaviors, evaluate tumor innervation, and test opioid effects on immunosuppression.

HPV-positive OPSCC patients reported significantly less pain at diagnosis and had lower PNI incidence compared to HPV-negative patients. In a cohort of 268 patients, only 29 (10.8%) reported opioid use at diagnosis. However, the opioid-use group had significantly decreased five-year survival probability; in adjusted analysis, opioid use was independently associated with worse survival (HR=3.60; 95%CI:1.40-9.28). In our mouse model, HPV-positive (mEERL) and HPV-negative (MOC2) tumors induced comparable orofacial nociceptive behaviors, and mEERL tumors were innervated by peripheral nerves, contradicting clinical observations. Preliminary TIL analysis is still underway, however, initial data suggests a trend toward opioid induced immunosuppression with increased FOXP3 Tregs.

These data suggest that pain and opioid use may serve as prognostic markers for worse outcomes in HPV-positive OPSCC. However, the mEERL model fails to recapitulate reduced pain and innervation observed clinically. Further work is warranted to clarify causality and develop appropriate preclinical models for this population.

Funding:1R01DE033473-01A1 NNS, T32 NS086749 NSA, no disclosures

From Cholesterol Load to Cholesterol Signal: LYCHOS-Driven mTORC1 Activation as a Mechanism of Residual Cardiovascular Risk

Ajam, Ali^{1,2}; Zhang, Xiangyu^{1,2}; Peroumal, Doureradjou^{1,2}; Liu, Ziyang^{1,2}; Razani, Babak^{1,2}

¹Department of Medicine and Vascular Medicine Institute, University of Pittsburgh School of Medicine and UPMC

²Pittsburgh VA Medical Center

Atherosclerosis is a chronic inflammatory disease driven by excess lipids and maladaptive immune responses. Although aggressive lowering of low-density lipoprotein (LDL) reduces cardiovascular risk, many patients continue to experience events, suggesting that LDL may promote immune dysfunction through mechanisms independent of circulating LDL levels. We investigated whether LDL-derived cholesterol acts as an intracellular signaling molecule in human monocytes and macrophages through the lysosomal cholesterol sensor LYCHOS (GPR155), a regulator of mTORC1.

We hypothesized that LDL-derived cholesterol activates monocyte mTORC1 signaling through LYCHOS, thereby impairing autophagy-associated pathways and promoting pro-atherogenic immune phenotypes. CD14⁺ monocytes were isolated from hypercholesterolemic participants and normocholesterolemic controls (n=10/group). LYCHOS expression was quantified by qPCR and mTORC1 signaling assessed by phosphorylation of S6 kinase and S6. Mechanistic studies were performed in human macrophages using cholesterol depletion–repletion paradigms with LDL stimulation and LYCHOS siRNA knockdown. Autophagy-associated pathways were evaluated by LC3 puncta formation and TFEB nuclear localization. Complementary in vivo studies were performed in ApoE^{-/-} mice subjected to Western diet and chow re-feeding to examine leukocyte and plaque macrophage signaling.

Hypercholesterolemic participants exhibited significantly increased monocyte mTORC1 signaling compared with normocholesterolemic controls. Importantly, LYCHOS expression was markedly elevated in monocytes from hypercholesterolemic individuals, whereas normocholesterolemic controls demonstrated substantially lower LYCHOS levels. In human macrophages, LDL exposure activated mTORC1, increased lysosomal mTOR recruitment, reduced TFEB nuclear localization, and decreased LC3 puncta, consistent with suppression of autophagy-associated pathways. LYCHOS knockdown significantly blunted LDL-induced mTORC1 activation. Preliminary studies in myeloid-specific LYCHOS-deficient mice further suggest reduced atherosclerosis.

These findings identify a conserved cholesterol–LYCHOS–mTORC1 signaling axis linking LDL-derived cholesterol to immune dysfunction in human monocytes. Elevated LYCHOS expression in hypercholesterolemic individuals suggests that cholesterol sensing pathways may contribute to residual cardiovascular risk beyond LDL levels and represent a potential target for precision immunometabolic therapies in atherosclerosis.

Investigating the Role of ER stress in Ovarian Cancer Quiescence and Chemoresistance

Arrigo, Austin¹; Hijazi, Amjad¹; Roberts, Alex¹; Ahmed, Noor¹; Buckanovich, Ronald^{1,2}

¹Department of Internal Medicine and Magee-Womens Research Institute, University of Pittsburgh

²Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Hillman Cancer Center, University of Pittsburgh

Ovarian Cancer (OvCa) is one of the most common and deadly gynecological cancers diagnosed in women, leaving patients with a poor 5-year survival rate of less than 50%. OvCa tumors are highly treatment resistant, and nearly 70% of patients have recurrence, further contributing to poor patient outcomes. One severely understudied mechanism of treatment resistance and recurrence is quiescence. Quiescence is a unique cellular state where cells can reversibly exit and re-enter the cell cycle, making them highly resistant to chemotherapy and able to seed recurrent tumors. Therefore, identifying factors to specifically target quiescent OvCa (qOvCa) may help reduce treatment resistance, recurrence, and promote better patient outcomes. To this end, we performed an ER Bio-ID analysis followed by mass spectrometry and discovered a unique Quiescent Associated Secretory Profile (QuASP). Nearly 30% of all factors identified in the QuASP are involved in protein folding or ER stress response. Additionally, we found that induction of quiescence led to an increase in ER stress response factors on the mRNA and protein levels. Interestingly, we found that induction of ER stress by pan-HSP70 inhibitor (vernalix), resulted in a significant reduction in cell growth, without increasing cell death. Furthermore, vernalix treatment promoted chemoresistance and increased the G0 population. Together, these data demonstrate that ER stress may be an important component in quiescence induction. To abrogate these protective ER stress effects in qOvCa, we treated cells with inhibitors targeting key ER stress response pathways, PERK, ATF6, and IRE1 α . Interestingly, treatment with either drug alone was not toxic to actively proliferating OvCa cells. However, when combined with quiescence inducers, we found increased sensitivity resulting in decreased cell viability and altered QuASP expression. Therefore, developing a combination therapy that targets quiescent associated ER stress may reduce qOvCa treatment resistance, tumor recurrence, and provide a better outcome for OvCa patients.

Spatial Transcriptomic Profiling Reveals Atorvastatin Modulates Tumor Microenvironment to Suppress Breast Cancer Metastatic Outgrowth

Atale, Neha¹; Chen, Jie⁴; Isett, Brian⁴; Bao, Riyue^{4,5}; Meyer, Ernest M.⁴; Wells, Alan^{1,2,3*}

¹Department of Pathology, University of Pittsburgh

²Research and Development Service, Pittsburgh VA Health System

³Cell Biology Program, Hillman Cancer Center, University of Pittsburgh

⁴University of Pittsburgh Medical Center Hillman Cancer Center

⁵Department of Medicine, University of Pittsburgh

*** Author to whom correspondence should be addressed**

A major worldwide health concern, metastatic breast cancer is still an aggressive and treatment-resistant disease. Cancer cells escape from the primary tumor, travel to the bloodstream (disseminated tumor cells, or DTCs), and eventually make their way to distant organs and settling there as dormant cells. These can remain dormant for many years and even decades evading any treatment for the primary tumors. They can then become active (through second Epithelial-to-Mesenchymal transition) and have been known to mainly occur through the interaction between these dormant cells and the environment around them in the host organ. The key mechanisms and triggers for this change are yet unknown, however, previous research has shown that statins, especially Atorvastatin, that have been historically used in regulation of cholesterol through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) can reduce the proliferation of metastatic secondary tumors while having minimal effect on primary tumors. In this study, we used Spatial Transcriptomics to understand the cellular configuration in and around the tumor sites in untreated and atorvastatin treated liver tissue samples in mice using Nanostring CosMX Spatial Molecular Imager. Moreover, it altered the cellular configuration around the tumor boundary (Tumor Associated FOVs) by reducing the proportion of cancer associated fibroblasts, hepatic stellate cells, and M1/M2 like macrophages. This distribution remained unchanged in the non-tumor regions for the samples. The atorvastatin treated samples also showed significant downregulation of key bioactive matrix genes in these specific cell types suggesting a potential cellular pathway driven mechanism through which atorvastatin inhibits the metastatic proliferation. These results provide a rationale for further investigating the mechanisms of atorvastatin and provide insights that help us create novel long term adjuvant drugs to manage breast cancer recurrence and improve patient survival.

Elucidating the Distinctive “Eye-print” among Neurodevelopmental Disorders

Avni, Inbar¹; Patterson, Christina²; Dinstein, Ilan^{3,4}; Behrmann, Marlene^{1,5}

¹Department of Ophthalmology, University of Pittsburgh

²Department of Pediatrics, Division of Child Neurology, University of Pittsburgh

³Psychology Department, Ben Gurion University of the Negev, Beer Sheva

⁴Azrieli National Centre for Autism and Neurodevelopment Research, Ben Gurion University of the Negev

⁵Department of Psychology, Carnegie Mellon University

Although decades of research have led to distinctive phenotypes for different neurodevelopmental disorders, a systematic set of features for the objective classification of these disorders has not been developed. Here, in the context of a basic and translational science investigation, we used gaze behavior, a ubiquitous, easily accessible behavior that both tracks alterations in developmental trajectories and provides insight into the underlying neural computations and representations, to distinguish between these disorders. We acquired detailed measures of the gaze profile, or “Eye-print” while 98 participants with autism, epilepsy and childhood hemispherectomy, along with matched controls viewed a short engaging movie that repeated at least twice. To fully characterize the groups, we extracted a set of 54 dependent measures: oculomotor (e.g. saccade count, velocity), spatial (e.g. visual field eccentricity, direction), temporal (e.g. dwell time, inter- and intra-subject correlations over time), and content-based (e.g. frame-by-frame analysis of social ROIs and basic properties like spatial frequency) features, as well as pupillometry metrics. A unique set of individual and combined measures differentiated the Eye-print of the patients from matched controls, and from the three patient groups. All patient groups differed in gaze variability compared to controls. As expected, children with autism showed atypical gaze to social stimuli. In contrast, children with hemispherectomy exhibited asymmetric oculomotor deficits, with a greater number, longer distance, and longer duration of saccades toward the intact visual field, and compensatory peripheral stimulus fixation patterns towards the impaired visual field. Children with epilepsy demonstrated atypical spatial saccades and a peripheral stimulus fixation bias. Taken together, the 54 features enabled significant group classification, with clustering analysis revealing greater similarity between the epilepsy and autism groups relative to the hemispherectomy group. These findings permit an understanding of the idiosyncratic phenotypes of neurodevelopmental alterations and elucidate fundamental psychological and neural signatures that have diagnostic leverage.

Ubiquitin C-terminal Hydrolase L1 (UCHL1): A novel modifier of *FUS*-ALS mediated toxicity

Bajaj, Anjali^{1,2}; Anderson, Eric^{1,2}; Kour, Sukhleen^{1,2}; Patil, Snehal^{1,2}; Goswami, Anand³; Pandey, Udai^{1,2}

¹Department of Pediatrics, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center

²Children's Neuroscience Institute, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center

³Department of Neurology, Center for Motor Neuron Biology and Disease, Eleanor and Lou Gehrig ALS Center, Columbia University

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by loss of upper and lower motor neurons, leading to progressive muscle weakness and atrophy. Although most cases are sporadic, roughly 10% have genetic causes, of which mutations in the RNA-binding protein fused in sarcoma (*FUS*) underlie a clinically severe, juvenile-onset subset of ALS. Previously, we established a *Drosophila* model of *FUS*-associated ALS that recapitulates several disease features, including motor and synaptic dysfunction, cytoplasmic aggregation, and neurodegeneration. Using this model, we performed an unbiased genetic screen to identify modifiers of *FUS* toxicity. Here, we report identification of the deubiquitinase *UCHL1* as a novel and potent genetic modifier of *FUS*-driven neuropathology and motor dysfunction *in vivo*. *UCHL1* is a highly abundant neuronal deubiquitinase that plays a critical role in ubiquitin-proteasome-mediated protein turnover. RNAi-mediated knockdown of *UCHL1* in animals expressing human *FUS* strongly suppressed retinal degeneration, improved motor function, and extended survival. By contrast, *UCHL1* overexpression exacerbated these phenotypes in our *FUS in vivo* model, further supporting the proof-of-concept. Mechanistically, *UCHL1* appears to act on cytoplasmic *FUS* aggregates, as RNAi-knockdown in a juvenile-onset P525L *FUS* fly model reduced *FUS* inclusion burden and restored viability. Importantly, patient-derived tissues carrying the *FUS* mutation showed increased *UCHL1* levels, accompanied by cytoplasmic co-localization of *UCHL1* with *FUS* aggregates in motor neurons, indicating disrupted ubiquitin homeostasis in the disease. Similar observations were made in HEK cells transfected with *FUS* WT and mutant plasmids under sodium arsenite stress. Together, these results identify *UCHL1* as a modifier of *FUS* toxicity and highlight ubiquitin-dependent protein turnover as a point of intervention in *FUS*-mediated ALS.

Sex-Dependent Effects of Adolescent Stress on Basolateral Amygdala to Medial Prefrontal Cortex Inhibitory Plasticity

Balasubramanian, Hari Deva Muthu¹; Grace, Anthony¹

¹Departments of Neuroscience, Psychiatry, and Psychology, University of Pittsburgh

Exposure to adversity during adolescence significantly increases the risk for psychiatric disorders, including schizophrenia and depression, with growing evidence suggesting that both the timing of stress and biological sex critically determine neurobiological outcomes. The basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) form a reciprocally inhibitory circuit central to stress responsivity, the maturation of which extends across adolescence and represents a key window of vulnerability. Prior work from our laboratory demonstrated that in male rats, adolescent stress (postnatal day [PD] 31–40) induces precocious adult-like long-term depression (LTD) in BLA–mPFC inhibitory plasticity within 1–2 weeks, whereas adult stress disrupts this established connectivity, indicating that stress timing fundamentally determines whether corticoamygdalar plasticity is accelerated or impaired. Complementarily, sex-dependent sensitive windows have been identified wherein males exhibit selective vulnerability to dopamine dysregulation following prepubertal stress, while females demonstrate heightened susceptibility during postpubertal periods. Despite these advances, whether adolescent stress differentially impacts BLA–mPFC inhibitory plasticity in females remains uncharacterized, representing a critical gap in understanding sex-biased stress vulnerability. To address this, female Sprague-Dawley rats were subjected to the identical combined footshock and restraint stress protocol (PD 31–40), and BLA–mPFC inhibitory synaptic plasticity was assessed using in vivo extracellular electrophysiology at 1–2 weeks and >6 weeks post-stress. Preliminary findings reveal a striking divergence from the male pattern: naïve females already exhibit robust BLA–mPFC LTD at the 1–2 week timepoint, suggesting this inhibitory pathway reaches functional maturity earlier in females than in males. Furthermore, adolescent stress neither accelerated nor disrupted this plasticity in females at either timepoint, in sharp contrast to stress-exposed males. Collectively, these preliminary findings suggest sex-dependent trajectories of BLA–mPFC circuit maturation and stress sensitivity, potentially contributing to divergent psychiatric vulnerabilities following adolescent adversity. Continued data collection will establish statistical significance and inform sex-specific frameworks for understanding stress-related neurodevelopmental psychopathology.

Enhancing GFAP-Based Prediction of Amyloid Pathology in Alzheimer's Disease with Additional Biomarkers: Insights from a Multi-Cohort Study

Balogun, Wasiu G.¹; Boots, Theresa E.²; Zeng, Xuemei²; Farinas, Marissa F.²; Nafash, Michel N.²; Shi, Ruyu¹; Emanuel, James E.²; Bedison, Margaret³; Matan, Cristy⁴; Nadkarni, Neelesh K.⁵; Gogola, Alexandra⁴; Shaaban, C. Elizabeth⁶; Aizenstein, Howard J.¹; Tudorascu, Dana L.¹; Pascoal, Tharick A.¹; Villemagne, Victor L.¹; Berman, Sarah B.⁵; Ikonovic, Milos D.¹; Sweet, Robert A.¹; Kofler, Julia⁷; Snitz, Beth E.⁵; Kamboh, M. Ilyas³; Saeed, Anum⁸; Reis, Steven E.⁸; Gildengers, Ariel¹; Cohen, Ann D.¹; Lopez, Oscar I.⁵; Karikari, Thomas K.¹

¹Department of Psychiatry, University of Pittsburgh

²Western Psychiatry Hospital, University of Pittsburgh Medical Center

³Department of Human Genetics, University of Pittsburgh

⁴Department of Radiology, University of Pittsburgh

⁵Department of Neurology, University of Pittsburgh

⁶Department of Health Promotion and Development, University of Pittsburgh

⁷Department of Pathology, University of Pittsburgh

⁸Department of Cardiology, University of Pittsburgh

GFAP has emerged as a leading plasma biomarker for astrogliosis. However, its diagnostic utility may be enhanced when combined with other biomarkers. This study investigates which biomarkers, when paired with GFAP, can provide a more comprehensive understanding of AD progression. We included 456 participants from 5 independent community- and clinical-based cohorts utilizing A β -PET and tau-PET. Plasma biomarkers were measured using the NULISAsTM CNS Disease Panel 120. We analyzed the interaction of each biomarker in the panel with GFAP according to pathology status and cohort. Logistic regression (75/25 training/testing split with 10-fold cross-validation) was used to predict A (or T) status. Adjustments were made for cohort, age, race, gender, education, and APOE4 status when available. Cognitive status was assessed via global CDR scores and analyzed via Kruskal Wallis and Dunn Tests. The AUC values and p-values demonstrated significant interactions: for A-status, eight biomarkers showed a significant interaction with GFAP in more than one cohort (IL2, SAA1, SLIT2, PDGFRB, CX3CL1, CRH, CXCL1, and PTN). When the cohorts were combined, 41 and 16 biomarkers showed significant interaction with GFAP for predicting A and T status, respectively. Among these, 5 biomarkers (p-tau217, CALB2, p-tau231, PARK7, and TIMP3) increased the accuracy of predicting A status compared to GFAP alone, while three biomarkers (p-tau217, CALB2, and IL5) enhanced T status prediction. Notably, the interaction of p-tau217 with GFAP was significant for both A and T statuses, and the combination of these biomarkers improved prediction accuracy for A status (combined: AUC 0.809; 95% CI: 0.764-0.854; GFAP alone: 0.722; 95% CI: 0.664-0.779; p-tau217 alone: 0.779; 95% CI: 0.728-0.830). For T status, the prediction accuracies for the multi-markers were comparable to those of single biomarkers. Our findings indicate that combining plasma GFAP with other AD biomarkers improves alignment with brain amyloid pathology and may enhance therapeutic trial assessments.

Topical tissue nanotransfection with Ascl1-Brn2-Myt1l mitigate thermal hypoesthesia and rescue small fiber neuropathy in murine diabetic peripheral neuropathy

Bommana, Raghunath Reddy¹; Palakurti, Ravichand¹; Roy, Sashwati¹; Sen, Chandan K.¹; Khanna, Savita¹

¹McGowan Institute for Regenerative Medicine, Department of Surgery, University of Pittsburgh

The emergent field of vasculogenic, neurogenic and neurotrophic skin reprogramming offers promise to address skin pathologies such as diabetic peripheral neuropathy (DPN). Peripheral neuropathies, characterized by the selective loss of small-diameter sensory nerve fibers lead to thermal sensory deficits. The current work tested the effects of direct in vivo reprogramming in diabetic mice (db/db) skin employing tissue nanotransfection based delivery of transcription factors Ascl1, Brn2, and Myt1l (TNT_{ABM}) to restore function in DPN. Single application of TNT_{ABM} to the dorsal and thigh skins rescued the loss of intraepidermal nerve fiber density (IENFD) in the plantar skin. Small sensory fibers showed a significant recovery of the peptidergic marker CGRP as well as regeneration markers GAP43 and PGP9.5 within the plantar skin epidermis and PRPH density in the sciatic nerve. High-resolution multiplex imaging validated that the regeneration was selective for small sensory fibers. Markers for large-diameter fibers remained unchanged. TNT_{ABM} treatment restored thermal sensitivity, which is mediated by small fibers, but had no effect on large fibers. TNT_{ABM}-induced genes associated with thermal nociception, including TRPV1 and CaV2.2, while the mechanosensitive channel PIEZO2 remained unaffected. The therapy specifically elevated NGF, a key neurotrophin for small fiber survival. Collectively, these findings establish minimally invasive topical neurotrophic reprogramming of the skin in vivo as a promising therapeutic strategy to manage small fiber neuropathies.

The influence of social factors of health on asthma exacerbations in *All of Us*: A Work-in-Progress

Borkowski, Vera^{1,4}; Deng, Zhirui^{2,4}; Koleck, Theresa^{3,4}

¹Department of Health and Community Systems, University of Pittsburgh

²Office of Research and Scholarship, University of Pittsburgh School of Nursing

³Department of Health Promotion and Development, University of Pittsburgh

⁴University of Pittsburgh School of Nursing

Introduction: Uncontrolled childhood asthma often leads to chronic school absenteeism, resulting in lower educational attainment. The effects of absenteeism and lower educational attainment can last beyond childhood, negatively affecting health in adulthood. Conversely, higher educational attainment lowers risk of certain conditions, e.g. Alzheimer's Disease and diabetes, however, the effects of educational attainment for adults diagnosed with asthma in childhood has not been investigated. Thus, our objective is to determine the influence of educational attainment on asthma exacerbations in adults diagnosed with asthma in childhood, within the context of other social factors of health. We hypothesize that a bidirectional relationship exists between educational attainment and asthma.

Methods: This is a retrospective cohort study of adult participants with diagnosed with asthma during childhood enrolled in the All of Us Research Program. We identified adult participants who reported being diagnosed with asthma between 0-17 years of age on the Personal and Family Health History survey (N= 20,369). We identified instances of asthma exacerbations using *All of Us* electronic health record data, specifically exacerbation diagnosis codes, emergency visits or hospitalizations related to asthma, or use of systemic corticosteroids. Education attainment will be obtained from The Basics survey question, "What is the highest grade or year of school you completed?" Additional social factor of health (e.g., neighborhood cohesion, social support, food insecurity, and housing quality), informing the social and physical environment of an individual, were obtained from the Social Factors of Health survey.

Work-in-Progress / Next steps: We plan to perform multivariable logistic regression to investigate the relationship between educational attainment and asthma exacerbations, controlling for other social factors of health. We anticipate the strength of associations to vary by category of educational attainment.

VIP Signaling in the Prefrontal Cortex Promotes Alcohol Motivation and Reshapes Cortical Output Circuits

Dakota, Brockway¹; Samuel, Boehm¹; Nilah, Jordan¹; Max, Joffe¹

¹Department of Psychiatry, University of Pittsburgh

Alcohol use disorder is characterized by maladaptive decision-making and persistent alcohol seeking, yet the circuit mechanisms within the prefrontal cortex that drive alcohol motivation remain poorly understood. Vasoactive intestinal peptide (VIP) interneurons are powerful regulators of cortical network activity through disinhibitory circuit motifs and VIP neuromodulatory signaling. However, the role of VIP interneurons in alcohol-related behaviors has not been established. We investigated how VIP signaling in the prelimbic prefrontal cortex regulates alcohol motivation and prefrontal circuit activity. Whole-cell patch clamp electrophysiology revealed that VIP directly depolarizes VIP interneurons, suggesting that VIP signaling can amplify VIP interneuron activity within cortical circuits. Consistent with this mechanism, intoxicating doses of systemic alcohol administration increased calcium activity in VIP neurons measured using fiber photometry in vivo. To determine whether VIP neuron activity contributes to alcohol seeking, we manipulated VIP neurons during operant alcohol self-administration. Chemogenetic activation of VIP neurons significantly increased motivation for alcohol under progressive ratio schedules, indicating that VIP neuron activity promotes alcohol-seeking behavior. In addition to regulating interneuron activity, VIP signaling differentially modulated layer 5 pyramidal neurons depending on projection subtype. Electrophysiological recordings revealed that VIP excitation enhanced excitability in extratelencephalic neurons while suppressing action potential firing in intratelencephalic neurons, suggesting that VIP signaling may bias prefrontal output toward specific downstream circuits. Together, these findings identify VIP interneurons as a previously unrecognized regulator of alcohol motivation and suggest that alcohol recruits VIP signaling to reshape prefrontal cortical output.

ANGPTL3 Inhibition by Evinacumab Improves Insulin Resistance and Cardiac Lipid Metabolism to Prevent HFpEF in GCN5L1 KO Mice

Bugga, Paramesha¹; Manning, Janet R.¹; Stoner, Michael W.¹; Mooli, Rajagopal Reddy²; Ramakrishnan, Sadeesh²; Mullett, Steven³; Scott, Iain^{1*}

¹Cardiology Division, Department of Medicine, University of Pittsburgh

²Endocrinology and Metabolism, Department of Medicine, University of Pittsburgh

³Pharmacology and Chemical Biology, Department of Medicine, University of Pittsburgh

GCN5L1, a mitochondrial acetyltransferase essential for regulating cardiac lipid metabolism, and ANGPTL3, a lipoprotein lipase inhibitor driving cardiometabolic disease progression, remain unexplored in HFpEF pathogenesis. Here, we investigate the mechanistic roles of cardiac-specific GCN5L1 deficiency and ANGPTL3 inhibition in a high-fat diet/L-NAME-induced HFpEF model. WT and cardiac-specific GCN5L1 KO mice received HFD/L-NAME for 15 weeks to promote HFpEF development. Evinacumab (10 mg/kg/week) was given in weeks 11–15. We assessed glucose tolerance, echocardiography (EF, FS, E/e', and IVRT), organ morphometry (heart and liver), H&E, MT staining, and heart transcriptomics/metabolomics. We assessed the ANGPTL3 expression and LPL activity in GCN5L1 knockdown (KD) cardiac AC16 cells, and in cardiac-specific GCN5L1 knockout (KO) mice. We demonstrated that systolic heart function was preserved in either genotype. However, diastolic function (i.e., increased E/e', IVRT, and cardiac fibrosis) were significantly increased in WT HFD/L-NAME-treated mice compared to WT chow mice, and this was further exacerbated by cardiac-specific GCN5L1 deletion. Multi-omics approach revealed that cholesterol synthesis and lipid metabolism pathways were significantly perturbed in the hearts and livers of GCN5L1 KO HFpEF mice compared to WT HFpEF mice. In particular, GCN5L1 KO mice displayed significantly elevated hepatic steatosis, suggesting a novel heart-to-liver metabolic pathway. GCN5L1 KD AC16 cells and KO mice exhibited a significant upregulation of ANGPTL3, which drives hyperlipidemia by inhibiting LPL and subsequent systemic lipid uptake. Evinacumab treatment, a new FDA-approved monoclonal antibody against ANGPTL3, reduced insulin resistance in WT HFpEF mice, and resulted in a moderate improvement in diastolic cardiac function.

Cardiac-specific GCN5L1 deficiency accelerates HFD/L-NAME-induced HFpEF by upregulating ANGPTL3 expression, thereby driving insulin resistance, hepatic steatosis, cardiac interstitial fibrosis, and diastolic dysfunction. Evinacumab, an ANGPTL3 monoclonal antibody, rescues GCN5L1 KO mice from HFpEF by inhibiting ANGPTL3, enhancing LPL activity, correcting lipid metabolism, insulin resistance, and reducing fibrosis.

Denoising functional MRI of the marmoset brain with BM4D-PC

Campos, Vinicius P.¹; Szczupak, Diego¹; LjungQvist-Brinson, Lovisa^{1,3,4}; Zimmermann Rollin, Isabela²; Corigliano, Michael R.^{1,3,4}; Dieckhaus, Laurel⁵; Schaeffer, David J.^{1,2,3,4}; Silva, Afonso^{1,2,3,4}

¹Department of Neurobiology, University of Pittsburgh

²Department of Bioengineering, University of Pittsburgh

³Center for Neuroscience, University of Pittsburgh

⁴Center for the Neural Basis of Cognition, University of Pittsburgh

⁵Department of Cardiology, University of Pittsburgh

Introduction:

Image post-processing denoising algorithms are valuable tools for overcoming the low-SNR limitation of high-resolution fMRI (Faes et al., 2024). We recently proposed BM4D-PC (Campos et al., 2025), a denoising method for diffusion MRI that achieves superior noise removal compared with traditional methods. Here, we adapted and applied BM4D-PC to task BOLD fMRI data from the common marmoset (*Callithrix jacchus*).

Methods:

A single-shot 2D GE-EPI was used to acquire task BOLD fMRI on a 9.4T BRUKER scanner with the following parameters: TE/TR = 15/2000 ms; FA = 35; BW = 200KHz; resolution = 0.5 x 0.5 mm; slice thickness = 1mm; matrix size = 84x35x20; Intervolume delay 1450ms. Using MRI-compatible headphones (STAX SR-003, Stax Ltd., Japan) and PsychoPy (Peirce, 2007), we presented 30 randomized 40-s blocks of either a repeated single-exemplar marmoset “Phee” call (1450 ms, every 2s) or silence.

For benchmarking, we denoised the data using both BM4D-PC and NORDIC (Moeller et al., 2021).

After denoising, fMRI processing was performed using custom Bash scripts following the marmoset brain connectome preprocessing pipeline (Schaeffer et al., 2022). Briefly, images were phase-encoded corrected in FSL (Jenkinson et al., 2012; Andersson and Sotiropoulos, 2016), aligned in space, despiked, had the first 5 TRs removed, temporally low-pass filtered (freq $\leq$ 0.1 Hz), and motion-censored in AFNI (Cox, 1996). Processed images were then registered to the Marmoset Brain Mapping (MBM) template (Liu et al., 2018) using Advanced Normalization Tools (ANTs) (Tustison et al., 2021).

Results:

Both NORDIC and BM4D-PC improved fMRI data quality, with BM4D-PC producing the highest tSNR and resulting in larger activation clusters and greater statistical significance ($p < 0.01$) in the auditory cortices.

Conclusion:

BM4D-PC achieved state-of-the-art results in noise removal, demonstrating its promise as a superior denoising algorithm for fMRI data. Future work will include resting-state fMRI and simulated datasets.

TFF3 Expression Patterns in the Thyroid and Its Association with Thyroid Cancer Differentiation

Cetinkaya Hosafci, Gamze T.¹; Fornal, Ian J.¹; Nikitski, Alyaksandr V.¹; Nikiforov, Yuri E.¹

¹Department of Pathology, University of Pittsburgh

Trefoil factor 3 (TFF3) is a secretory peptide with protective roles in various tissues. In thyroid, its function and expression changes in benign and cancer cells remain unclear. This study aimed to investigate expression patterns of TFF3 in normal thyroid and different types of thyroid cancers and its potential relations with expression of thyroid differentiation genes.

RNA sequencing was used to evaluate *TFF3* expression and its correlations with thyroid differentiation markers. qRT-PCR was performed to compare *TFF3* expressions among normal thyroid, Graves' disease, subtypes of PTC (RAS-like and BRAF-like) and ATC samples. Immunohistochemistry (IHC) assessed localization and expression pattern of TFF3 in thyroid samples. Iodine uptake assay evaluated the impact of siRNA-mediated *TFF3* silencing in thyroid cells.

RNAseq analysis of 85 thyroid samples (10 normal thyroid, 5 BRAFV600E PTC, 39 RAS PTC, 22 PDTC and 9 ATC) revealed a positive association between *TFF3* expression and thyroid dedifferentiation score (TDS). Among TDS genes, thyroid peroxidase (*TPO*) and thyroglobulin (*TG*) showed highest positive correlations with *TFF3* expression ($r=0.36$, $p=0.0006$; $r=0.44$, $p<0.0001$, respectively). Moreover, *TFF3* expression also correlated with BRAF-RAS score ($r=0.28$, $p=0.007$). As compared to normal thyroid, qRT-PCR demonstrated significantly higher *TFF3* expression in Graves' disease ($p=0.0056$), and progressively lower expression in PTC RAS-like ($p=0.0013$), PTC-BRAF like ($p=0.0006$) and ATC ($p=0.0006$). Silencing *TFF3* significantly reduced iodide uptake ($p<0.0001$) compared to both controls. By IHC and IFA, TFF3 was found to be highly expressed in thyroid follicular cells, showing diffuse cytoplasmic staining and colloid positivity.

In conclusion, *TFF3* has strong expression in normal thyroid and in Graves' disease both by IHC and mRNA analyses, with reduced and eventually lost expression in thyroid cancers, in close correlation with cancer dedifferentiation. Association between *TFF3* expressions, thyroid cell differentiation, and iodine uptake suggests that TFF3 may play a role in thyroid differentiation and iodine metabolism and may represent a previously unrecognized marker of thyroid differentiation.

Identifying User Needs in Powered Mobility and Manipulation Device Users: Implications for Addressing Challenges in Mobility and Activities of Daily Living

Chandrashekhar, Raghuveer¹; Satpute, Shantanu Avinash¹; Park, Sangmi¹; Flaugh, Owen^{1,2}; Sivakanthan, Sivashankar¹; Cooper, Rosemarie¹; Cooper, Rory A.^{1,2}; Candiotti, Jorge Luis^{1,2}

¹Human Engineering Research Laboratories (HERL), University of Pittsburgh

²Department of Bioengineering, University of Pittsburgh

Introduction: Over 12% of US adults report mobility-related disabilities, with 41% of long-term care users requiring caregiver assistance for at least one activity of daily living(ADL), driving \$490B in annual healthcare expenditures projected to double by 2040. Powered Mobility and Manipulation Devices(PMMDs) support independence and community participation for ~500,000 US users, growing at 4.5% annually. Yet current PMMDs offer limited indoor/outdoor functionality, with users facing difficulties navigating uneven terrain, architectural barriers, and burden and complexity of performing ADLs with existing PMMDs; These challenges are further compounded by chronic disabilities/impairments that evolve over a lifetime, emphasizing the need for adaptable assistive technology. This study aims to identify user needs and challenges when performing daily tasks with PMMDs.

Methods: A cross-sectional mixed-methods study was conducted using focus groups and a Qualtrics survey. Eligible participants were ≥ 18 years old, used a PMMD as their primary means of mobility, and reported difficulties performing everyday manipulation tasks. Those unable to speak/understand English or lacking internet/device access were excluded.

Results: Twenty-six participants (12 males, 14 females; age range 20–89, median 55 years) completed the study. 73% (n=19/26) of the participants reported ADL impairment by, and 46% (12/26) reported both mobility and ADL impairment. Uneven terrain and sidewalks were the most common barriers affecting 84% (22/26) and 76% (20/26) respectively. Furthermore, 57%(15/26) were unable to overcome curbs or 1–2 step entrances, and only 11% (3/26) reported doing so without difficulty. Additionally, 30% (8/26) – 50% (13/26) reported complete dependence for dressing, meal-preparation, toileting, and self-hygiene.

Discussion/Conclusion: This study highlights everyday mobility and instrumental ADL challenges faced by PMMD users consistent with the current evidence base. These findings underscore the limitations in current technologies and establish a foundation for next-generation PMMD design incorporating greater sensing and actuation capabilities to support mobility-related ADLs. However, limited racial and ethnic diversity restricts generalizability.

Targeting DPP4 to Enhance T Cell Infiltration and Improve Cancer Immunotherapy

Chen, Shangyu^{1,2,*}; Li, Shichen^{1,2,*}; Hua Zhang^{1,2}; Li, Song^{1,2}

¹Center for Pharmacogenetics, Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh

²University of Pittsburgh Cancer Institute, University of Pittsburgh

*These authors contributed equally to this work

Cancer immunotherapy has transformed cancer treatment, yet its efficacy remains limited by a “cold” tumor immune microenvironment (TIME) characterized by poor T cell infiltration. Induction of immunogenic cell death (ICD) has been shown to improve T cell infiltration by promoting the release of T-cell-recruiting chemokines, such as CXCL10. However, the overall outcome is limited by dipeptidyl peptidase IV (DPP4), a serine protease that degrades CXCL10 and related chemokines. We herein report that ICD-inducing chemotherapeutic agents transcriptionally upregulate DPP4 in cancer cells, potentially acting as a negative feedback mechanism that dampens the magnitude of immune response. To improve the delivery of both DPP4 inhibitor and ICD-inducing agent while minimizing the systemic off-target side effect, we developed a hyaluronic acid (HA)-dendrimer nanocarrier for tumor-targeted co-delivery of the two therapeutic agents. HA is a natural ligand for CD44 that is overexpressed on both tumor cells and tumor endothelial cells, enabling precise targeting. In preclinical animal tumor models, this strategy significantly enhanced T cell infiltration, anti-tumor immunity, and the overall therapeutic efficacy while minimizing systemic toxicity. Our work establishes a novel immunochemotherapy platform that synergizes chemotherapy and immunotherapy via targeted DPP4 inhibition, offering a promising strategy to improve clinical outcomes in immunotherapy-resistant cancer.

Microbiome Composition and Dynamics in Tracheal Aspirates during Chronic Critical Illness

Cheng, An-Chi¹; Gupta, Anshika¹; Cooper, Vaughn S.^{2,3}; Zemke, Anna C.¹

¹Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Medicine, University of Pittsburgh

²Department of Microbiology and Molecular Genetics, University of Pittsburgh

³Center for Evolutionary Biology and Medicine, University of Pittsburgh

Patients requiring prolonged mechanical ventilation are at high risk for respiratory infections that frequently involve coinfections with multiple pathogens. However, the airway microbiome of individuals with tracheostomies during chronic infection remains inadequately characterized, complicating antibiotic decision-making and infection management. This study aims to characterize the composition and longitudinal dynamics of the airway microbiome in patients with tracheostomies, especially for the effects of antibiotics. Tracheostomy aspirates were collected from patients recovering from critical illness with a tracheostomy in a long-term acute care hospital in Pennsylvania, USA. A total of 164 samples were obtained from 51 patients, including 18 patients with more than three sampling time points. Full-length 16S rRNA gene sequencing was performed using Oxford Nanopore Technology. Filtered reads were taxonomically classified using the Emu pipeline with a custom database integrating rrnDB and the NCBI 16S RefSeq database. Across all samples, the most frequently detected bacterial species were *Pseudomonas aeruginosa* (68.7%), *Corynebacterium striatum* (58.5%), *Mycoplasma salivarium* (29.9%), *Staphylococcus aureus* (28.6%), and *Stenotrophomonas maltophilia* (27.2%). *P. aeruginosa* was detected in 27.3% of patients during the first week of sampling and increased to 47.3% in subsequent weeks. In contrast, *S. aureus* was detected in 18.2% of patients during both the first and later weeks. Detection of *S. maltophilia* increased from 10.9% in the first week to 23.6% in subsequent weeks. PERMANOVA analysis of Bray–Curtis beta diversity demonstrated that airway microbiome composition was strongly patient-specific, whereas week-to-week temporal variation had a minimal and non-significant effect. Ongoing analyses will investigate factors driving microbiome dynamics and pathogen persistence, including the impact of antibiotic treatment, and potential co-evolution of dominant species during prolonged critical illness. These findings aim to inform antimicrobial resistance patterns and guide more effective antibiotic treatment strategies for patients with tracheostomies.

Mitotic Nanotubes: A Novel Mechanism for Maintaining Cell-cell Communication During Mitosis

Cooper, Anthony¹; Cetin-Ferra, Selma¹; Yonosh, Katie A.²; Hinton Jr., Antentor³; Marshall, Andrea G.³; Faeder, James R.²; Murray, Sandra A.¹

¹**Department of Cell Biology, University of Pittsburgh**

²**Department of Computational and Systems Biology, University of Pittsburgh**

³**Department of Molecular Physiology and Biophysics, Vanderbilt University**

During mitosis communication is reduced as the gap junction protein connexin 43 (Cx43) is redistributed from plaques on the plasma membrane to cytoplasmic vesicles. It was recently reported that Cx43 is also stored in transient, actin-based mitotic nanotubes that connect mitotic cells to their neighbors. However, the trafficking dynamics of Cx43 within mitotic nanotubes remain unclear. Furthermore, whether these Cx43-containing mitotic nanotubes play a role in intercellular communication between mitotic cells and their neighbors is not known. It is hypothesized that Cx43-positive structures are delivered to the ends of mitotic nanotubes where they facilitate gap junction-mediated cell-cell communication. Live-cell confocal microscopy was used to assess the dynamics of mitotic nanotube-associated Cx43 structures, and fluorescence-based functional assays were used to measure intercellular communication via mitotic nanotubes. Within mitotic nanotubes, Cx43 structures were dynamically remodeled and were either transferred to the cytoplasm of one of the associated cells or deposited at the ends of the mitotic nanotubes where they were positioned to potentially allow gap junctional communication. Mitotic nanotubes facilitated the communication of the gap junction-permeable molecule calcein but at a reduced rate compared to gap junctions between interphase cells. Blockage of gap junction channels and knockdown of Cx43 inhibited cell-cell transfer, supporting that communication via mitotic nanotubes occurs in a gap junction-dependent manner. Knockdown of Cx43 impaired nanotube formation and communication, while inhibition of Rho kinase impaired cell rounding and nanotube elongation but increased communication. Together, these findings uncover mitotic nanotubes as a novel mechanism for maintaining gap junction-mediated communication during cell division and provide mechanistic insight into the roles of Cx43 trafficking and actin remodeling in intercellular communication in the dividing cell.

Neural Correlates of Stress-Induced Alcohol Seeking: Goal-Directed vs Habit-Like Behaviors

Correll, Erika¹; Bartholomey, Megan²; Torregrossa, Mary¹

¹Department of Psychiatry, University of Pittsburgh

²Department of Neuroscience, Allegheny College

One theory of addiction is posited as a transition from goal-directed to habitual behavior. Alternatively, the incentive salience sensitization theory suggests that addictive behavior arises from brain circuitry that mediates incentive salience (IS), the motivational drive to pursue a reward-associated cue despite minimal derived pleasure. Ascribing IS to a reward-predictive cue enables the cue to elicit reward-seeking actions and craving. The tendency to attribute incentive salience, measured as sign-tracking during a Pavlovian conditioned approach (PavCA) task, may increase the risk of developing addictive behaviors. Interestingly, sign-tracking non-drug reward cues is indicative of greater probability of relapse. Exposure to alcohol cues and stress can be additive potent drivers of relapse. Stress is a factor known to modulate the control of instrumental actions such that goal-directed behavior shifts to favor habitual behavior. Dysfunctions in this modulation governed by stress may promote the development of psychiatric disorders such as addiction.

Studies have demonstrated that the lateral habenula (LHb) modulates ventral tegmental area (VTA) dopamine (DA) release during goal-oriented behaviors. Importantly, VTA DA release is required for sign-tracking. Our lab has found that rats are sensitive to pharmacological stress via yohimbine (YOH) to drive increased cue-induced alcohol seeking; however, it's unclear how stress modulates sign-tracking behaviors.

This study aims to identify LHb activation patterns associated with goal-directed alcohol reinstatement and habit-like sign tracking following pharmacological stress. Goal-directed rats were trained to self-administer alcohol followed by instrumental extinction. These rats were then exposed to cue-induced reinstatement of ethanol seeking following injection of YOH (1.25 mg/kg) or vehicle. Habit-like rats performed PavCA for an alcohol reinforcer for 7 days. On the 8th day, rats were administered YOH (1.25 mg/kg) or vehicle prior to the PavCA task. All brains were collected 90 minutes after the reinstatement session or final PavCA session for cFos immunohistochemistry.

Switching Telomerase Function: Dissociation from RNA TERC Enables Non-Canonical Telomerase Functions in Osteogenic Reprogramming in Calcific Aortic Valve Disease

Cuevas, Rolando A.¹; Panzhu, Ilias¹; Mackenzie, Kenny¹; Gakii, Nina¹; Behzadi, Parya¹; Cynthia St. Hilaire¹

¹Vascular Medicine Institute, Department of Medicine, School of Medicine, University of Pittsburgh

Calcific aortic valve disease (CAVD) is the most prevalent heart valve disorder and lacks effective medical therapies. Inflammation is a recognized driver of CAVD progression, yet the initiating molecular events that commit valvular cells to an osteogenic fate remain incompletely defined. Telomerase is a multi-subunit complex best known for telomere maintenance, with telomerase reverse transcriptase (TERT) and the RNA component TERC forming its core. TERT is best known for its telomere maintenance functions; however, it can also exhibit transcriptional regulatory functions. The mechanism regulating TERT's switch from its telomeric role to non-canonical co-transcriptional regulatory functions is not known. Our prior work found that TERT initiates molecular events that drive valvular cell osteogenic differentiation. Mechanistically, TERT promotes RUNX2 expression via interaction with Signal Transducer and Activator of Transcription 5 (STAT5). To enable mechanistic dissection, we used AI-assisted structural modeling to determine TERT-STAT5 interactions and found that TERT's RNA-binding domain, classically associated with interaction with TERC, also interfaces with STAT5, raising the possibility that dynamic TERT/STAT5 association and physical separation from TERC reflect the functional transition from telomeric activity to non-canonical co-transcriptional regulation. We validated these predictions by developing a real-time tool to visualize TERT/STAT5 interactions in living cells and by determining the dissociation and subcellular distribution of TERT and TERC during osteogenic differentiation. Together, these findings shed light on the underlying mechanism driving osteogenic reprogramming and calcification of valve cells and on a potential therapeutic target to blunt valvular calcification.

TransOT: Interpretable LLMs for finding common signatures of fibrosis across tissues

Cundiff, Mary¹; Hara, Mika²; Sriram, Varsha²; Liu, Chloe¹; Rahimikollu, Javad¹; Davuluri, Hitesh¹; Natarajan, Niranjana²; Das, Jishnu¹

¹Department of Immunology, University of Pittsburgh

²Division of Rheumatology and Clinical Immunology, Department of Medicine, University of Pittsburgh

Fibrosis, characterized by excessive deposition of extracellular matrix proteins (ECM), is associated with the etiology of most chronic inflammatory diseases, and can affect numerous vital organs - heart, liver, kidney, and lung. Progressive fibrosis results in replacement of healthy tissue with ECM, compromising organ structure and causes functional impairment. Fibrotic diseases account for 45% of all-cause adult mortality. Although the underlying causes that drive fibrosis may vary based on tissue and disease, ECM-producing myofibroblasts are the key drivers of fibrosis. While studies have identified molecular and cellular signatures of fibrosis within individual organs, conserved mechanisms of fibrosis across tissues have not been analyzed, despite fibrosis primarily driven by TGFB/SMAD signaling. To address this gap, we combine interpretable machine learning approaches developed by our group to single-cell foundation models to systematically compare fibrotic expression profiles across heart, lung and kidney. Specifically, we couple SLIDE to Geneformer (a single-cell foundation model leveraging a transformer architecture) and generate expression-informed gene embeddings, allowing us to capture high-dimensional representations of fibrotic cellular programs. By constructing context-specific probability distributions from gene expression matrices and employing optimal transport methods, we compute a measure of sharedness between clustering structures across tissues. This framework enables the identification of conserved fibrotic signatures, providing insight into fundamental molecular pathways underlying fibrosis irrespective of tissue context. Understanding these cross-tissue signatures will help develop generalizable pan-tissue therapeutic targets for fibrosis.

Rational Design of Selective IL-2-based Activators for CAR T Cells Using AlphaFold3 and Physics-Informed Machine Learning

Dahmani, Larbi Zakaria¹; Banerjee, Anupam²

¹Department of Computational & Systems Biology, University of Pittsburgh

²Department of Biochemistry and Cell Biology, Laufer Center for Physical & Quantitative Biology, College of Arts & Sciences, and School of Medicine, Stony Brook University

Recombinant human Interleukin-2 (rhIL-2, Aldesleukin) is used in immunotherapy for metastatic melanoma and renal cell carcinoma. Low-dose IL-2 has been investigated for administration after adoptive T cell transfer to enhance CAR T expansion and sustain effector function. However, systemic IL-2 can cause severe toxicities and promote expansion of regulatory T cells (Tregs). Previous attempts at mitigating cytokine-mediated side effects involved isolating CAR T cell signaling from endogenous immune responses by developing IL-2/IL-2R β based selective ligand–receptors systems. Expressing these variant orthogonal (ortho)IL2-R β receptors in CAR T cells and supplying variant orthoIL-2, was shown to dramatically improve selectivity in CAR T cell expansion and anti-tumoral potency in a leukemia mouse model. This study describes the computational design of synthetic orthogonal cytokine receptor-ligand systems based on the scaffolds of the human canonical IL-2 and IL-2R β . Leveraging state-of-the-art AlphaFold3 (AF3) structure prediction capabilities and a physics-informed constrained sequence generator (CSG), the pipeline generates, filters and ranks sets of putative orthoIL-2/orthoIL-2R β mutant designs. Variants displaying minimal predicted off-target interactions and enhanced in target contacts are prioritized for structural modelling. Top designs showed outstanding AF3 structural and interfacial quality metrics ipTM and pTM, with averages between cognate pairs of 0.724 ± 0.05 and 0.770 ± 0.042 , respectively. All in-silico hits showed ipTM < 0.5 for non-cognates, indicating a good likelihood of orthogonality. Additionally, putative hits showed high levels of predicted structural fidelity to wild-type (WT) human IL-2/IL-2R β (PDB: 2ERJ), with an average structural root-mean-square deviation (RMSD) of 0.843 ± 0.375 Å. Altogether, the results support the putative foldability and selective affinity of top-ranking mutants displaying metrics close-to or within experimental reference range. Finally, strengths and limitations are discussed, alongside the experimental implications of coupling a constrained protein design pipeline to the discovery and validation of selective binders based on naturally occurring scaffolds.

Dyadic Digital Health Interventions for Persons with Cognitive Impairment or Dementia and Their Family Caregivers: A Systematic Scoping Review

de Rosa, Cristina¹; Zhang, Yuchen¹; Kim, Woonkyung¹; Callan, Judith¹; Koleck, Theresa¹; Lingler, Jennifer H.²

¹School of Nursing, University of Pittsburgh

²Betty Irene Moore School of Nursing, University of California, Davis

Introduction

User-facing digital technologies, such as mobile applications or videoconferencing, have enhanced or replaced in-person health intervention delivery. Innovative solutions must be accessible, acceptable, and scalable to meet the unique needs of adults with cognitive impairment, Alzheimer's disease, or other dementias and the family caregivers who support them. Addressing individuals with dementia and family caregivers together as dyads may provide additional benefits. The purpose of this scoping review is to identify and describe dyadic digital health interventions for adults with cognitive impairment or dementia and their family caregivers.

Methods

This review followed guidelines from the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR). PubMed, CINAHL, and PsycINFO were searched for terms related to digital health technologies and dementia caregiving dyads. To be eligible for inclusion, both individuals with any form of cognitive impairment and family caregivers interacted with a technology component of a health intervention. Any trial design reporting health-related or feasibility, acceptability, or usability outcome was eligible. Records were managed using review software Rayyan. Two reviewers independently screened records at the title and abstract stage and full-text stage, with discussion with a third reviewer to resolve discrepancies. Data were extracted and cross-checked for accuracy.

Results

Out of 1,639 records retrieved, 73 ultimately met inclusion criteria. Included articles represented individuals with all levels of self-reported or diagnosed cognitive impairment and family members including spouses/partners, adult children, and other relationships. Study designs included randomized controlled trials, single-arm trials, and pilot studies. Interventions targeted various health-related objectives, like improving well-being, supporting self-management, and facilitating health communication. Studies included active components for both dyad members and multiple physical, cognitive, psychosocial, and feasibility outcomes. Design and implementation of future digital health interventions should be tailored to build on existing strengths and meet their dyadic health needs.

Traffickome: an inference engine for directed membrane trafficking and signaling crosstalk

Deshpande, Rushikesh H.¹; Pinilla Macua, Itziar¹; Gygi, Steven²; Sorkin, Alexander¹

¹Department of Cell Biology, University of Pittsburgh School of Medicine

²Department of Cell Biology, Harvard Medical School

Membrane trafficking regulates receptor signaling by controlling its intracellular locations, intensity, duration and outcomes. However, no computational framework exists to quantitatively link directed trafficking transitions with the signaling pathways of receptors and their downstream effectors from quantitative molecular data. Here we introduce Traffickome, an inference engine that scores 10 directed trafficking transitions across 8 cellular compartments using 792 curated trafficking proteins, integrated with 2,474 signaling proteins spanning 16 pathways. A permutation-based scoring engine classifies each transition as specifically active, broadly active, or silent, and names the molecular drivers. An execution-layer filter of 452 machinery proteins enables route-specific scoring from phosphoproteomics by restricting transition scores to coats, SNAREs, tethers, and motors. Traffickome predicted cargo (receptors and their signaling complexes) fate with 95.8% accuracy across 13 cargoes and 9 experimental modalities, and the in-silico perturbation analysis correctly predicted gene knockout effects across five independent datasets, including the recycling collapse caused by VPS35 loss. Applied to phosphoproteomics of head-and-neck squamous cell carcinoma cells stimulated with epidermal growth factor, the combined trafficking–signaling analysis captures the collapse of kinase-dependent endosomal signaling in the presence of Erlotinib, an inhibitor of the EGF receptor kinase activity, and the emergence of retromer-mediated recycling of kinase-inhibited receptors. We further applied Traffickome to 1,030 tumors from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) spanning ten cancer types. This analysis identified a co-activation of secretory transport and TGFβ/SMAD signaling that predicted patient survival in pancreatic, lung and brain cancers, and was confirmed by spatial co-expression analysis in oral squamous cell carcinoma tissue sections (Visium spatial transcriptomics). Notably, pancreatic cancer patients with co-elevated secretory trafficking and TGFβ scores had half the median survival of other patients, and neither score alone was prognostic. Traffickome runs in the browser and as a Python pipeline, requiring no installation.

Defining the mechanism of IFN γ mediated regulation of intestinal epithelium during environmental enteric dysfunction (EED)

Dev, Gagan¹; Liu, Taohua¹; Bhattacharjee, Amrita¹; Hand, Timothy¹

¹Department of Immunology, University of Pittsburgh

Environmental enteric dysfunction (EED) is a major contributor to childhood morbidity and mortality in low- and middle-income countries and is characterized by villous atrophy, immune infiltration, and impaired intestinal barrier function. To investigate the immunopathogenesis of EED, we developed a murine model combining malnutrition with colonization by adherent-invasive *E. coli*. EED mice exhibited increased frequencies of IFN γ -producing CD8 T cells, expansion of ROR γ ⁺ Tregs, and substantial loss of absorptive enterocytes in the small intestine.

Neutralization of IFN γ markedly improved villous architecture and disease severity, establishing IFN γ as a central mediator of pathology. CD8 T cell-specific deletion of IFN γ similarly ameliorated villous damage, identifying CD8-derived IFN γ as the dominant pathogenic source. In contrast, deletion of *Rorc* in Tregs exacerbated villous atrophy and increased CD8 T cell-derived IFN γ , demonstrating that ROR γ ⁺ Tregs restrain pathogenic CD8 responses. Mechanistically, MHCII deletion in *Rorc*⁺ antigen-presenting cells impaired induction of ROR γ ⁺ Tregs, unleashing CD8-derived IFN γ and worsening epithelial injury.

We further detected bacterial translocation in EED mice; intravenous ampicillin reduced bacterial dissemination, diminished IFN γ -producing CD8 T cells, and restored villous structure, implicating microbial translocation as the upstream trigger of CD8 activation. At the epithelial level, STAT1 signaling was elevated in EED. Conditional deletion of epithelial IFN γ receptor reduced villous atrophy, and pharmacologic JAK inhibition with ruxolitinib ameliorated disease severity.

Together, these findings define a pathogenic circuit in which translocating bacteria induce CD8 T cell-derived IFN γ that signals through epithelial IFN γ R-STAT1 pathways to disrupt intestinal stem cell function and villous integrity, while ROR γ ⁺ Tregs serve as critical regulators of this axis. Targeting microbial translocation or IFN γ signaling may represent therapeutic strategies for EED.

The Purpose of Service Placement: Does Potential Access Create a “Compliance Trap” for Older Adults Navigating Reentry?

Dickens, Jacinta¹

¹Department of Psychiatry, University of Pittsburgh

For older adults navigating reentry, the physical location of supportive services is a critical determinant of community reintegration. This study evaluates the "rationality of placement" for reentry agencies within California-Kirkbride, specifically focusing on the recent relocation of the Foundation of HOPE to 901 Pennsylvania Avenue. While the agency provides essential housing and legal referrals, its efficacy is tied to the environmental accessibility of the surrounding neighborhood. This research argues that the mere presence of an agency creates only potential access—a presumed marker of service availability that may not translate into realized access for the population it intends to serve.

Utilizing City of Pittsburgh Sidewalk Inventory data and Pittsburgh Regional Transit frequency, this research documents the physical barriers that separate residents from this central service hub. Analysis reveals a significant mismatch: while the neighborhood has a high density of low income housing, the infrastructure—characterized by poor sidewalk ratings and low-frequency transit—fails to meet City Code benchmarks for functional independence for older residents. By mapping these micro-barriers, this study demonstrates how the physical environment effectively nullifies the proximity of the service provider.

This research questions the purpose of placing referral-based services in a service desert where the physical environment acts as a gatekeeper. If an agency primarily offers referrals to off-site providers, the burden of navigation falls on a population with limited environmental control. Without integrated infrastructure improvements, the placement of reentry hubs in deteriorating urban interiors may create a “compliance trap”—where residents are mandated to engage with services that are physically unreachable—rather than a pathway to actualized support.

Voxel-wise Prediction of Chronological Brain Age from MRI-Derived Brain Morphology in the Common Marmoset Using Convolutional Neural Networks

Dieckhaus, Laurel¹; Campos, Vinicius P.²; Zhang, Bei²; Dubberley, Lauren ²; McGee, Emilee²; Szczupak, Diego²; Silva, Afonso C.^{2,3}

¹Department of Cardiology, University of Pittsburgh

²Department of Neurobiology, University of Pittsburgh

³Department of Bioengineering, University of Pittsburgh

Analyses of age-related brain changes often rely on regional summary measures, which can obscure spatially heterogeneous effects. To address this limitation, we developed a voxel-wise convolutional neural network (CNN) framework to examine age-related brain morphology in the common marmoset. This enables a data-driven, spatially unconstrained analysis of age-related brain changes.

The dataset comprised 280 structural MRI scans from 161 unique common marmosets spanning a broad age range (4–188 months; 93 males, 68 females), with approximately half of the scans acquired longitudinally. CNNs were trained to predict chronological age directly from logarithm-of-the-determinant Jacobian maps derived from local deformation fields. Five-fold cross-validation was performed at the subject level to ensure that scans from the same individual were not included in both training and testing folds. CNN performance was evaluated using the Pearson correlation between predicted and chronological age at the time of the scan. Saliency maps were generated to quantify voxel-wise contributions to CNN-based age prediction.

CNN performance was evaluated using cross-validated correlations between predicted and chronological age (mean $r = 0.866$ with a MAE=13.09 months). Group-averaged saliency maps revealed spatially heterogeneous brain regions contributing to age prediction. These age-salient regions provide targets for further anatomical and statistical characterization.

This work establishes the feasibility of CNN-based voxel-wise analysis for marmoset MRI studies, providing a complementary framework for characterizing brain aging. Predictive performance may be further improved through data augmentation and the integration of additional MRI contrasts to provide complementary structural information. In future applications, similar CNN-based models may be used to distinguish normative aging from disease-related deviations or to predict behavioral outcomes that capture functionally relevant variability beyond chronological age alone.

Multiplexed Fc array assays reveal a strong Th1-biased response following vaccination of mice with live-attenuated Rift Valley fever virus Δ NSs Δ NSm

Divens, Ashley M.^{1,2}; Mueller Brown, Karina^{1,2}; Fatma, Farheen³; Allen, Cadence³; Price, David A.⁴; Leung, Daisy W.⁴; Amarasinghe, Gaya K.³; McElroy, Anita K.^{1,2}

¹**Division of Pediatric Infectious Diseases, Department of Pediatrics, University of Pittsburgh**

²**Center for Vaccine Research, University of Pittsburgh**

³**Department of Pathology and Immunology, Washington University School of Medicine**

⁴**Department of Medicine, Washington University School of Medicine**

Rift Valley fever virus (RVFV) is a mosquito-borne arbovirus that infects humans and livestock, primarily in Africa and the Middle East. In humans, RVFV typically presents as a self-limiting febrile illness, but can also cause hemorrhagic fever, hepatitis, or encephalitis in more serious cases. The live-attenuated RVFV Δ NSs Δ NSm vaccine provides durable protection against RVFV in animal models, but our understanding of the quality of RVFV-specific antibodies remains incomplete. Most studies only report total IgG binding titers and/or virus neutralization following Δ NSs Δ NSm vaccination, providing a partial view of antibody function that fails to consider the role of Fc effector function in mediating protection. To address this knowledge gap, we developed a multiplexed assay where the nucleocapsid protein (N) and glycoproteins (Gn/Gc) were conjugated to magnetic beads. Serum from vaccinated mice was incubated with the beads to capture antigen specific antibodies and various detection reagents were used to quantitate isotype, FcR binding, and complement binding capabilities. Serum from C57BL/6 mice vaccinated with high or low doses of RVFV Δ NSs Δ NSm were assessed using this assay. The predominant isotype of N- and Gn/Gc-specific antibodies following vaccination, regardless of dose or time post-vaccination, was IgG2c. This finding is consistent with a strong Th1 response following Δ NSs Δ NSm vaccination, as has been previously shown. The magnitude of response for N-specific antibodies was also two-fold higher than that of Gn/Gc-specific antibodies. Additionally, N- and Gn/Gc-specific antibodies demonstrated binding to the activating Fc receptors Fc γ RI and Fc γ RIV, indicating their potential to induce Fc effector functions such as antibody-dependent cellular cytotoxicity. There was also detectable binding of RVFV-antibodies to C1q, suggesting they could activate the complement system to induce opsonization. Overall, this study establishes the Fc profile of the highly protective RVFV Δ NSs Δ NSm vaccine and can serve as a benchmark for future RVFV vaccine platforms.

Biological Age Modeling Using Electronic Health Records

Edakalavan, Smitha¹; Nouraie, Seyed Mehdi¹

¹Division of Pulmonary, Allergy, Critical Care Medicine, Department of Medicine, University of Pittsburgh

Background

Chronological age is a strong predictor of morbidity and mortality, yet individuals of the same age can differ substantially in physiological health. Biological age measures may better capture this heterogeneity and improve risk stratification. Electronic health records (EHRs) offer a scalable source of clinical data for estimating biological age, although challenges such as data harmonization, unit inconsistencies, and missingness must be addressed.

Objective

To evaluate the feasibility and clinical relevance of estimating biological age from EHR data using Levine PhenoAge and the Klemmera-Doubal Method (KDM).

Methods

We conducted a retrospective study of adult patient encounters from a large health system in western Pennsylvania (2010–2025). Laboratory data were harmonized across naming conventions and units, implausible values were excluded, and missing data were handled using a two-step imputation strategy (30-day forward/backward window followed by multivariable chained equations). Biological age and age acceleration (residuals from regression on chronological age) were computed using both models. Associations with comorbidities, mortality, and 30-day readmission were evaluated.

Results

The dataset included 151,762 encounters from 2,615 patients (mean age 57; 59% female). KDM showed tighter alignment with chronological age, while PhenoAge demonstrated greater dispersion. Both measures correlate with chronological age ($r=0.61$). PhenoAge acceleration was associated with multiple comorbidities, including chronic kidney disease, diabetes, heart failure, and respiratory failure. In Cox models, PhenoAge (HR 1.037), KDM (HR 1.042), and chronological age (HR 1.060) were all significantly associated with mortality, with PhenoAge achieving the highest concordance (0.72). Both measures were also significantly associated with increased 30-day readmissions.

Conclusions

Biological age can be derived from EHR data with appropriate preprocessing. Despite limitations in biomarker availability and model assumptions, these measures are associated with clinically meaningful outcomes and may support risk stratification and personalized care in hospital settings.

Targeting Anterior Gradient-2 Driven Programs to Reverse Immune Exclusion in Breast Cancer Liver Metastasis

Elhaw, Amal^{1,2}; Phan, Angelica^{1,2}; Hintelmann, Tom^{1,2}; McCue, Lauren^{1,2}; Liu, Jia-Jun¹; Liu, Silvia¹; Williams, Michelle^{1,2}

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh

²Cancer Biology Program, UPMC Hillman Cancer Center, University of Pittsburgh

Breast cancer liver metastasis (BCLM) is a major driver of breast cancer mortality and one of the least immune-infiltrated metastatic sites. Consequently, most BCLM lesions are ineligible for treatment with current immunotherapy approaches. Defining the molecular basis of immune exclusion in BCLM is therefore critical for identifying new biomarkers of response and developing therapeutic strategies beyond current immune checkpoint-based approaches.

Using NanoString-based transcriptomic profiling of 20 human BCLM specimens, our group found that BCLM is more immunologically heterogeneous than previously appreciated, with distinct immune-hot and immune-cold subgroups. Through this analysis, we identified the secreted protein Anterior Gradient-2 (AGR2) as the most significantly upregulated gene in immune-cold BCLM. Multispectral immunofluorescence conducted in both the discovery cohort and an independent validation cohort (n = 40 total BCLM) further demonstrated an inverse association between tumor AGR2 expression and CD8⁺ cytotoxic T-cell abundance in the BCLM microenvironment. Based on these findings, we hypothesized that tumor-intrinsic AGR2 promotes T-cell exclusion. Indeed, we found that AGR2-high tumors exhibit reduced T-cell infiltration and activation.

To define the mechanisms underlying this phenotype, I am employing both established murine models and highly translational systems, including some of the only BCLM PDOs cultured in physiologically relevant liver-on-a-chip platforms. Using these models, I found that AGR2-high tumors secrete higher levels of fibronectin, suggesting a mechanism by which AGR2 may establish a microenvironment that restricts T-cell infiltration. I also demonstrated that AGR2 expression is induced by endoplasmic reticulum (ER) stress. Building on this, I am currently evaluating FDA-approved ER stress-targeting agents for their ability to disrupt this pathway and reverse AGR2-driven immune exclusion.

Together, this work identifies a novel and therapeutically actionable ER stress/AGR2/fibronectin axis that promotes immune exclusion in BCLM and provides a strong foundation for strategies aimed at overcoming immune evasion at this uniquely resistant metastatic site.

X-chromosome-wide meta-analysis identifies genetic associations with orofacial clefts

Erdogan-Yildirim, Zeynep¹; Weinberg, Seth M¹; Shaffer, John R^{1,2}; Simpson, Claire^{3,4}, Beaty, Terry H⁵; Bailey-Wilson, Joan³; Ruczinski, Ingo⁶; Marazita, Mary L^{1,2,7}

¹Center for Craniofacial and Dental Genetics, Department of Oral and Craniofacial Sciences, University of Pittsburgh

²Department of Human Genetics, University of Pittsburgh

³Computational and Statistical Genomics Branch, National Human Genome Research Institute, National Institutes of Health

⁴Department of Genetics, Genomics and Informatics, University of Tennessee Health Science Center

⁵Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

⁶Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health

⁷Clinical and Translational Sciences, School of Medicine, University of Pittsburgh

Orofacial clefts (OFCs) are among the most common congenital defects and demonstrate sex-biased prevalence, with cleft lip with or without cleft palate (CL/P) occurring twice as often in males as in females. This pattern along with emerging evidence implicating X-linked genes in OFC etiology suggests that the X chromosome may be a plausible contributor to non-syndromic OFCs. Although several X-linked genes like *TBX22*, *MID1*, and *OFD1* are known to be causal for syndromic forms of OFCs, X-chromosomal markers implicated in non-syndromic OFC risk remains underexplored.

To address this gap, we conducted an X-chromosome-wide meta-analysis involving 12,055 individuals (5,407 females and 6,648 males) of multiethnic ancestry from three independent samples. The POFC1 and GENEVA samples were genotyped with the Illumina Human610-QuadV1_B BeadChip array and the POFC2 sample with the Illumina GDA-8v1-0 array. Additional genotypes were imputed using the TOPMed reference panel r3 via TOPMed Imputation Server and minimac4. Variants with poor imputation quality ($r_{sq} < 0.8$) were excluded. We performed sample-specific association testing: POFC1 and POFC2 were analyzed using logistic regression adjusting for sex, the principal components of ancestry, and relatedness using the GENESIS package, and the offspring-parent trios in the GENEVA sample using family-based association testing (FBAT).

We identified one statistically significant CL/P locus in the intergenic region between *EFNB1* and *PJAI* (chrX:69094776_G/T, $p = 7.03 \times 10^{-11}$) surpassing the genome-wide significance threshold of 5×10^{-8} . This locus maps 4kb away from a known OFC locus. We also observed suggestive associations within *USP9X* (chrX:41224614_C/T, $p = 2.82 \times 10^{-6}$), and in the intergenic region between *SHROOM4* and *BMP15* (chrX:50847556_A/G, $p = 9.86 \times 10^{-6}$). Deletions involving *EFNB1/PJAI* and loss-of-function mutations in *USP9X* result in syndromes which frequently involve OFCs.

These findings underscore the importance of X chromosome in non-syndromic OFC risk. Our future work aims to identify sex-specific X-linked contributors to CL/P to further enhance our understanding of sex-biased biological mechanisms.

Refining Ecological Momentary Assessment for Phantom Limb Pain

Falbo, Kierra¹; Rich, Tonya^{2,3}; Hansen, Andrew^{2,3}; Kummerfeld, Erich⁴; Loth, Katie³; Fisher, Lee⁵

¹Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh

²Rehabilitation and Engineering Center for Optimizing Veteran Engagement and Reintegration, Minneapolis VA Health Care System

³Department of Family Medicine and Community Health, University of Minnesota

⁴Institute for Health Informatics, University of Minnesota

⁵Department of Physical Medicine and Rehabilitation, University of Pittsburgh

Phantom limb pain (PLP) affects most people with amputation, but evidence for treatment efficacy is inconsistent. To better inform PLP treatment, improved assessment methods are needed. Ecological momentary assessment (EMA) is a systematic, real-time method for measuring highly variable outcomes and predictive factors for those outcomes. Our goal is to use EMA to identify person-specific factors affecting PLP to inform personalized treatment. We hypothesized that EMA and causal modeling would identify stable and meaningful relationships between PLP and other measured factors (e.g., sleep). This longitudinal, observational study includes two phases. In Phase I, individuals with amputation and PLP were recruited. Participants received text notifications to complete an online survey three times per day for 40 days. These EMA surveys measured current PLP and potentially associated factors (e.g., sleep, activity, mood). Causal modeling was used to analyze datasets and identify person-specific factors predicted to cause PLP. In Phase II, in addition to EMA surveys, participants will use wearable devices to measure sleep, activity, and other objective factors that may be predictive of PLP. Participant feedback on data collection and perception of personalized results will be obtained through semi-structured interviews. Seventeen participants (10 male, mean age: 51.7) completed Phase I with a mean survey response rate of 89.3%. Causal models with face validity were produced using EMA data. Most factors predicted to affect PLP were unique to individual participants. These results suggest that EMA and causal modeling are feasible for identifying factors affecting PLP. Additionally, PLP factors are largely person-specific. Introducing wearables in Phase II is expected to increase data accuracy and consistency, improving causal modeling. Participant feedback will inform future implementation work. Individualized assessment of PLP is necessary for effective clinical care. Identification of factors affecting PLP can provide information for evidence-based, patient-specific treatment recommendations through a precision medicine approach.

Being “On the Go” and Interrupting are Most Strongly Associated with ADHD Diagnosis in a National Sample of Preschoolers in the United States

Feldman, Julia S.¹; Schiff, Arianna²; Lindhiem, Oliver¹; Joseph, Heather M.¹; ECHO Cohort Consortium³

¹Department of Psychiatry, School of Medicine, University of Pittsburgh

²Department of Communication Sciences and Disorders, School of Communication, Northwestern University

³Office of the Director, National Institutes of Health

Many behaviors related to attention-deficit/hyperactivity disorder (ADHD) are commonly endorsed by caregivers of preschool-aged children, making it difficult to determine whether symptoms are inconsistent with developmental expectations – a core component of the diagnostic criteria. Understanding which ADHD symptoms are most strongly associated with current or future diagnosis can aid in identifying which children are at greatest risk and require additional support. The purpose of the study was to examine which ADHD symptoms at ages 3-5 are most strongly associated with an ADHD diagnosis. Data were drawn from the Environmental Influences on Child Health Outcomes (ECHO) Program, a large national dataset in the US. Children were included if their caregiver(s) completed at least 1 of 5 measures that assessed ADHD symptoms at age 3 ($N = 1,430$), 4 ($N = 1,453$), and/or 5 ($N = 2,029$) years. ADHD diagnosis was determined by medical record and/or caregiver report. A series of logistic regression models were conducted with each symptom included as the independent variable and ADHD diagnosis as the dependent variable. Bootstrapped 99% confidence intervals of odds ratios were compared across symptoms. Next, age and interactions between age and symptoms were added to each logistic regression model. All 10 symptoms assessed were significantly associated with an ADHD diagnosis. *Being “on the go”* and *interrupts or intrudes* were most strongly associated with diagnosis. Associations between symptoms and diagnosis did not significantly differ by age despite significant interactions for *difficulty with follow-through*, *fidgets*, and *interrupts or intrudes*. Results suggest that some symptoms may be more useful than others when determining if a preschool-aged child is likely to be diagnosed with ADHD. Providers should consider the context and impairment associated with the symptoms. Future work should build upon limitations to assess all 18 ADHD symptoms in children with and without ADHD diagnoses across developmental periods.

Exploring the terrestrial-aquatic connection and building long-term ecosystem resiliency in Pittsburgh

Fetters, Andrea M.¹; Shirey, Patrick D.¹

¹Department of Geology and Environmental Science, University of Pittsburgh

Terrestrial and aquatic ecological realms are connected within ecosystems, but anthropogenic disturbances, like mining and recreational facility development, can disrupt that connection via environmental pollution, threatening ecosystem function and stability. One of the most ecologically devastating consequences of mining is acid mine drainage. As groundwater carries acid mine drainage throughout an area, heavy metals that are in acid mine drainage can be toxic to aquatic life and accumulate in plant tissues, which can have cascading effects through both aquatic and terrestrial realms. Similarly, the construction of recreational facilities results in habitat destruction, and their maintenance requires the substantial use of pesticides and fertilizer that may pollute groundwater and negatively affect aquatic and terrestrial life. Despite the pervasive issues caused by anthropogenic disturbance, ecological restoration of impacted areas can be achieved, especially if restoration efforts involve community participation.

Using a before-after control impact design in greenspaces in Pittsburgh that were previously both mined and recreational areas, our work in progress investigates how 1) community participation in ecological restoration activities change individual perspectives on conservation; 2) active restoration influences the ecological responses of plant, microbe, fish, and macroinvertebrate communities; and 3) active restoration impacts soil and water quality. We predict that 1) individuals involved in our project will be inclined to participate in future ecological restoration efforts, 2) communities will be more diverse (functional) in areas undergoing active restoration relative to those that are not, and 3) active restoration will increase soil and water quality. Our work will strengthen the terrestrial-aquatic connection and promote long-term ecosystem resilience in Pittsburgh, and integrating scientific research and community participation will create sustainable partnerships across multiple perspectives. It also creates the opportunity to inform ecological restoration policy and lays the foundation for future work that will examine the movement of water and nutrients in recovering ecosystems.

Hypothalamic H3K9 methylation regulates central energy metabolism

Galgano, Luca¹; Gui, Yijun²; Wu, Yanan²; Yaman Hizdri, Öykü¹; Muthler, Isabella A¹; and Çakır, Işın¹

¹Division of Endocrinology and Metabolism, Department of Medicine, University of Pittsburgh

²Life Sciences Institute, University of Michigan

Obesity and its comorbidities, including diabetes and cardiovascular diseases, represent major global health challenges. Current treatments such as glucagon-like peptide 1 receptor agonists (e.g., semaglutide) have limitations, including lean mass loss, gastrointestinal side effects, and weight regain, highlighting the need for a deeper understanding of body weight regulation.

Epigenetic mechanisms, particularly histone post-translational modifications, regulate chromatin structure and gene expression. Histone methylation varies by residue and degree (mono-, di-, or tri-methylation); for example, histone 3 lysine 9 methylation (H3K9me) represses transcription. Histone modifications also underlie metabolic transcriptional memory, where early-life diet or environmental factors cause lasting gene expression changes that elevate metabolic disease risk. In energy balance, this memory manifests as compensatory responses restoring original weight after loss. However, histone modifier roles in hypothalamic feeding centers remain unclear.

Our data indicate: (1) previously obese mice rapidly regain weight, (2) obesity leaves persistent hypothalamic gene expression changes (“obesogenic transcriptional memory”), (3) diet-induced obesity disrupts hypothalamic chromatin marks, notably H3K9me, even after weight loss, (4) hypothalamus-specific knockdown of H3K9 methyltransferases SETDB1 (set domain bifurcated histone lysine methyltransferase 1), SETDB2, and EHMT2 (euchromatic histone lysine methyltransferase 2) in lean mice induces obesity-like H3K9me profiles and obesity, and (5) SETDB1/SETDB2 modulate leptin signaling.

We hypothesize that hypothalamic H3K9me controls body weight and represents a target for obesity prevention or treatment. These findings provide insights into hypothalamic epigenetic regulation of energy and glucose metabolism and suggest that small-molecule inhibitors of SETDB1/SETDB2/EHMT2 could modulate body weight set point in preclinical and clinical contexts.

Developing an attention-based breast dense tissue segmentation algorithm for multi-center, multi-vendor data

Ganjee, Razieh¹; Mustafaev, Tamerlan¹; Hossain, Md Belayat²; Zuley, Margarita¹; Lee, Juhun^{1,3}

¹Department of Radiology, University of Pittsburgh

²Department of Computer Science, Southern Illinois University Carbondale

³Department of Bioengineering, University of Pittsburgh

Purpose: Breast density, defined as the proportion of glandular (dense) to fatty tissue, is a key risk factor for breast cancer. Accurate dense tissue segmentation from mammograms is essential for reliable density assessment. However, variations in tissue appearance across imaging vendors and centers make robust segmentation challenging. This study proposes a deep learning model that incorporates global contextual information to improve dense tissue segmentation in multi-vendor and multi-center datasets.

Methods and Materials: We developed a hierarchical encoder–decoder architecture enhanced with adaptive spatial and channel-wise attention mechanisms integrated into skip connections. This dual-attention design enables effective modeling of long-range dependencies and intra-class variability in tissue appearance. The model was trained and evaluated on 1,500 “for-presentation” mammograms from 606 women, collected from three independent datasets (500 images each), including one from Asia (Siemens) and two from the United States (Hologic). Radiologist-validated dense tissue masks were used as ground truth. Performance was assessed using 5-fold cross-validation. Percent density (PD) derived from segmentation results was further used to classify mammograms into dense (BI-RADS C–D) and fatty (A–B) categories.

Results: The proposed model achieved strong agreement with ground truth, with an overall Dice score of 0.72 ± 0.17 . Performance was highest in dense breast cases, with Dice scores of 0.84 ± 0.10 , 0.83 ± 0.11 , and 0.80 ± 0.10 across the three datasets. For PD-based classification of fatty versus dense breasts, the model achieved an AUC of 0.96, which was slightly lower than ground truth-based PD (AUC = 0.98; DeLong’s $p = 0.004$) but remained highly comparable.

Conclusion: The proposed model effectively segments dense breast tissue across diverse datasets by leveraging spatial and channel-wise attention to capture appearance variability.

Clinical Relevance/Application: This approach enables consistent breast density assessment across centers and vendors, supporting improved risk evaluation in clinical practice.

Building Trust: The Perspectives of COVID-19 Vaccination Efforts among African American Community Health Workers in HHS Region 4

Gray, Cecily A.¹; Bodine Al-Sharif, Mary Ann²; Elope, Latesha³; Sims, Omar T.^{4,5,6}; Alang, Sirry¹

¹Department of Health and Human Development, University of Pittsburgh

²Department of Human Studies, University of Alabama at Birmingham

³Division of Infectious Diseases, University of Alabama at Birmingham

⁴Department of Gastroenterology, Hepatology and Nutrition, Cleveland Clinic

⁵Department of Quantitative Health Sciences, Cleveland Clinic

⁶Lerner College of Medicine of Case Western Reserve University, Cleveland Clinic

Viral outbreaks such as COVID-19 have historically and disproportionately impacted low SES communities and communities of color. African Americans were disproportionately impacted by COVID-19 compared to others. At the height of the pandemic, vaccination was critical to mitigating the impact of the highly transmissible SARS-CoV2 virus. Despite this, hesitancy contributed to poor vaccination rates among adults in the United States. However, African Americans were more likely to report hesitancy and hesitancy associated with medical mistrust and structural barriers. Since vaccination is multifaceted and context specific, factors influencing vaccination should be considered within the appropriate context. Study Purpose: The current study examines perspectives of COVID-19 vaccination efforts among African American Community Health Workers (CHWs) in HHS Region 4 through Critical Discourse Analysis (CDA) using an ecological framework. Results: Findings indicate equitable vaccination efforts empowered underserved communities. Moreover, findings imply equitable vaccination efforts improved trustworthiness of government and public health officials despite political tensions. Findings also revealed CHWs tend to trust COVID-related information provided by the CDC, which is associated with acceptance. Despite religious tensions around vaccination, “The Church” emerged as a trustworthy location to receive COVID-19 information and vaccination. Conclusion: This research highlights the importance of community-centered public health interventions and trustworthiness. CHWs hold established trust within marginalized communities. “The Church” is a trusted and uniquely positioned institution able to facilitate health interventions and deliver essential health-related services.

Onboard AI at the Orbital Edge: A Mission Debrief from STP-H7-CASPR

Gretok, Evan¹; Silbernagel, Linus¹; Wildenstein, Diego¹; Cannizzaro, Michael¹; Roffé, Seth¹; George, Alan¹

¹NSF Center for Space, High-Performance, and Resilient Computing (SHREC); Department of Electrical and Computer Engineering, Swanson School of Engineering

The Configurable and Autonomous Sensor Processing Research (CASPR) payload was developed by students and faculty at the NSF SHREC Space Center at the University of Pittsburgh. It was designed to conduct autonomous Earth observation and perform onboard sensor data processing with a suite of novel imagers and flight computers. This payload was flown as one of six experiments aboard the Department of Defense Space Test Program – Houston 7 (STP-H7) pallet, mounted externally to the International Space Station. CASPR was also the first flight of the Satlantis iSIM-90 small-form-factor, low-spatial-resolution, multispectral visible and near-infrared (VNIR) imager.

This poster will explore the day-to-day operations and challenges overcome by the CASPR payload. Successful on-orbit checkouts were performed on all sensing and compute modules. Students supported manual and autonomous Earth observation tasks from the CASPR ground station located in the NSF SHREC facility at the University of Pittsburgh. Most of the CASPR mission objectives were completed successfully. After two years of successful data collection, the pallet was relocated to a different ISS site. This prevented further Earth imaging; however, additional experiments on radiation-effects monitoring and onboard processing continued without interruption.

CASPR achieved significant milestones during its nearly four years in orbit. More than fifty thousand monochrome frames and 48 multi-spectral image bursts were captured using the iSIM-90. The low 3.0-meter-per-pixel spatial resolution of these images enabled observation of individual buildings, ships, aircraft, and even large road vehicles during flight. Numerous students developed and deployed applications for image processing, computer vision, classification, and compression onboard. Additionally, 449 single-event upsets and 32 multi-bit upsets were observed during nearly 10 device-years of operation across the CSP and both SSPs. Overall, CASPR demonstrated itself to be a highly successful student-driven payload and provided tremendous opportunities for both student learning and technical advancement for onboard flight computing.

SLC39A6-targeted therapy to restore drug sensitivity in CAF-protected HER2+ breast cancer using a microfluidic platform

Habli, Zeina¹; Li, Tiangyang¹; Poskus, Matthew¹; Zervantonakis, Ioannis^{1,2}

¹Department of Bioengineering, University of Pittsburgh

²Hillman Cancer Center, University of Pittsburgh

HER2-positive (HER2+) breast cancer accounts for 20–30% of all breast cancer cases, and although HER2-targeted therapies have substantially improved patient outcomes over the last decade, drug resistance remains a significant challenge particularly in the metastatic setting, where most patients relapse, and in over 15% of early-stage patients. Emerging evidence suggests that cancer-associated fibroblasts (CAFs) in the tumor microenvironment (TME) reduce treatment efficacy through paracrine signaling and extracellular matrix remodeling. A better understanding of tumor-fibroblast interaction mechanisms in a patient-specific manner is necessary to develop fibroblast-targeted combination therapies. We used a panel of HER2+ breast cancer cell lines and patient-derived organoids (PDOs) cocultured with CAFs in a novel 3D microfluidic platform that enables precise spatial control of CAF-PDO interactions in a physiologically relevant matrix. We performed transcriptomic analysis of the NOAH clinical trial dataset and HER2+ breast cancer cells cocultured with CAFs to identify genes enriched in fibroblast-rich tumors with residual disease and fibroblast-protected cancer models. In the clinical trial dataset, transcriptomic analysis revealed that SLC39A6 (LIV-1/ZIP6) expression is significantly elevated in samples with residual disease compared to samples with good response following HER2-targeted therapy. Furthermore, we found that high SLC39A6 levels are associated with poor patient survival. In addition, our data revealed that SLC39A6 was significantly upregulated at the protein levels in 4 out of 6 HER2+ breast cancer models under CAF coculture but not monoculture conditions. Additionally, targeting SLC39A6 with the antibody-drug conjugate Ladiratuzumab vedotin in combination with lapatinib induced tumor cell death in CAF-protected breast cancer PDOs. This work leverages state-of-the-art microfluidic models that better recapitulate the complex TME and defines SLC39A6 as a novel target to reverse HER2 therapy resistance. Furthermore, our findings using clinically relevant PDOs will inform the development of personalized treatment strategies and biomarkers to aid in identifying high-risk patients.

Increased Gp130 in Pancreas-Innervating Dorsal Root Ganglia is Associated with Mechanical Hypersensitivity in a Cerulein-Induced Mouse Model of Chronic Pancreatitis

Hong, Veronica¹; Fulton, Stephanie¹; Epouche, Ariel¹; Choi, Ashley¹; Jefferson, Mia¹; Babyok, Olivia¹; Aquirre, Paulina Cabada¹; Davis, Brian²; Saloman, Jami¹

¹Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh

²Department of Neurobiology, University of Pittsburgh

Chronic pancreatitis (CP) pain is a debilitating gastrointestinal symptom and a major driver of hospital readmissions, yet current therapies do not target the molecular mechanisms that sustain ongoing nociceptive and neuropathic pain. Clinical data from the PROCEED cohort demonstrate that circulating cleaved glycoprotein 130 (gp130) is a stable serum biomarker that remains associated with both nociceptive and neuropathic pain descriptors over time, despite dynamic changes in patients' sensory phenotypes. Gp130, the shared signal transducer for the interleukin-6 (IL-6) cytokine family, has been implicated in neuronal sensitization and mechanical hypersensitivity across inflammatory, cancer, and neuropathic pain models, and nociceptor-specific gp130 deletion reduces mechanical hypersensitivity. However, whether gp130 signaling in pancreas-innervating sensory neurons directly contributes to CP pain remains unknown. Using a repeated cerulein CP model, we observe robust abdominal mechanical and spontaneous pain-like hypersensitivity, assessed by abdominal von Frey testing and rearing activity, respectively. These behavioral changes are accompanied by increased gp130 mRNA in thoracic dorsal root ganglia that innervate the pancreas and coordinated upregulation of multiple IL-6 family cytokines in the pancreas by qPCR. Together, these clinical and preclinical data support the central premise that CP-related pain is driven, in part, by gp130-dependent sensitization of pancreatic afferents. Future studies will use Trpv1-Cre-mediated conditional gp130 deletion and small-molecule gp130 inhibition to define neuronal gp130 as a mechanistic contributor and candidate therapeutic target for non-opioid, mechanism-based analgesia in CP pain.

Pharmacological inhibition of MET impairs hepatocyte regeneration in a murine model of acetaminophen-induced liver injury

Jain, Siddhi¹; Mukherjee, Ranjan¹; Williams, Gillian¹; Orr, Anne¹; Bhushan, Bharat¹

¹Department of Pathology, Pittsburgh Liver Research Center, University of Pittsburgh

Background: Hepatocyte growth factor receptor (MET) is a central driver of liver regeneration after partial hepatectomy, yet its role in acetaminophen-induced liver injury, the leading cause of acute liver failure in the Western world, remains unclear. Our prior work using hepatocyte-specific MET-KO mice showed severe injury and high mortality; hence, the present study uses Capmatinib (CAP), a selective MET inhibitor, to pharmacologically evaluate MET's regenerative capacity during AILI.

Methods: Male C57BL/6J mice received either a moderate (300 mg/kg) or severe (500 mg/kg) overdose of APAP, followed by CAP (15 mg/kg) or vehicle treatment 2 hr later, bid up to 48 hr. Bulk and spatial transcriptomic data from ALF patients were analyzed, and freshly isolated primary human hepatocytes were treated with APAP $\pm$ CAP to assess cell death.

Results: Both the moderate and severe APAP overdose resulted in comparable peak injury across groups, with elevated levels of serum biomarkers and centrilobular necrosis. During the recovery phase, both overdose levels showed a decline in injury and necrosis in vehicle-treated mice but remained markedly higher in CAP-treated mice. The similar extent of peak injury allowed assessment of MET's role in regeneration independent of injury. MET inhibition markedly reduced key cell cycle markers (pERK1/2, Cyclin D1, and PCNA) at 24 and 48 hr, indicating impaired or delayed regeneration with CAP treatment following moderate or severe overdose. Furthermore, a comprehensive analysis of RNA sequencing datasets showed that APAP ALF patients exhibited upregulation of cell-cycle genes, linked to an active regenerative response and robust activation of the HGF/MET signaling axis. Moreover, inhibiting MET worsened APAP-induced hepatotoxicity in primary human hepatocytes, underlining the protective role of MET signaling in AILI.

Conclusion: Overall, MET inhibition notably suppressed liver regeneration and impaired recovery following AILI, especially after severe APAP overdose, emphasizing MET's crucial role in regenerative response.

NOX2 inhibition as a therapeutic to target microgliosis, amyloidosis, and cerebrovascular dysfunction in Alzheimer's disease

Jamus, Andzoa N.^{1,5}; Kim, Jun Kyoung²; Chang, Christine³; Peretin, Jenna⁴; Vazquez, Alberto⁴; Cifuentes-Pagano, Eugenia^{1,5}; Pagano, Patrick^{1,5}

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh

²Department of Neuroscience, University of Pittsburgh

³Department of Biological Sciences, Carnegie Mellon University

⁴Department of Radiology, University of Pittsburgh

⁵Vascular Medicine Institute, University of Pittsburgh

Alzheimer's Disease (AD) is the leading form of dementia, characterized by cognitive decline and physiological hallmarks like amyloid plaques, tau pathology, and neuroinflammation. A primary driver of this pathology is oxidative stress caused by aberrant reactive oxygen species (ROS), impairing vascular function, and exacerbating inflammation. One major source of ROS is NADPH Oxidase 2 (NOX2), a multicomponent, membrane-bound enzyme known to play a role in age-related neuronal and vascular damage. Thus, we hypothesize that inhibiting NOX2 will ameliorate AD pathology and vascular dysfunction. To inhibit NOX2, small molecule CPP11G was used, predicted to compete with NOX2 subunit p47, binding to the enzyme's core. APP-PS1 mice overexpress amyloid precursor protein (APP) that is cleaved into A β protein, and expresses GFP on microglia for tracking. Mice are treated with CPP11G, and pathology is analyzed at regular intervals until 18 months old. Physiological hallmarks of AD are analyzed via cranial window and use of 2-photon (2P) microscopy. A β plaque formation is visualized by Methoxy XO4, and plaque volume is quantified via MATLAB. Microglia, a major phagocytic immune cell of the brain, typically clears A β plaque formation. However, as disease progresses, microglia display an altered, less mobile morphology and activity, releasing increased pro-inflammatory cytokines. This microgliosis is visualized by GFP-expressing microglia complemented by sulfur rhodamine injection for vascular visualization. Changes in morphology, mobility, and vascular association are quantified via MATLAB. Vascular reactivity is also known to be compromised with AD progression, with decreased vasodilation over time, altering proper vascular function. CO₂-induced vasodilation is detected by laser doppler flowmetry (LDF) probing and wide-field microscopy at the cranial window, quantified via MATLAB. Here, we show preliminary data highlighting halted A β plaque formation, declined vascular reactivity, and microgliosis changes with and without CPP11G treatment. Future work includes *ex vivo* analysis of NOX2 complexation and ROS production.

Differential cysteine utilization facilitates shifts in energy metabolism, thereby determining SIV progression in nonhuman primate models

Johnson, Samuel D.¹; Jasinska, Anna J.²; Senthilkumar, Shreyaa³; Deaktor II, Charles¹; Daira, Mohammed¹; Kandasamy, Sudhapriya¹; Apetrei, Cristian^{2,3}; Pandrea, Ivona^{1,3}

¹Department of Pathology, University of Pittsburgh

²Division of Infectious Diseases, Department of Medicine, University of Pittsburgh

³Department of Infectious Diseases and Microbiology, University of Pittsburgh

Determinants for HIV/SIV disease progression remain incompletely understood. Like ART-naïve people with HIV (PWH), Asian-origin cercopithecines, including pig-tailed macaques (PTMs), experience disease progression, with some experiencing rapid progression. Conversely, SIV natural hosts, like African green monkeys (AGMs), resist immune dysfunction, including persistent CD4⁺ T cell loss, despite viremia. We leveraged metabolomics to determine mechanisms contributing to SIV progression or non-progression in these species.

PTMs and AGMs were infected with 300 TCID SIVsab. PTMs were retroactively stratified into normal (n=12) and rapid progressors (n=6). Infected AGMs (n=4) were compared to SIV-naïve AGMs (n=6). Metabolic profiling was performed by ultra-high-performance liquid chromatography/tandem accurate mass spectrometry (UHPLC/MS/MS). Plasma viral loads, cytokines, and cell counts were measured in PTMs.

Preceding infection, the greatest predictor of rapid progression in PTMs was higher cysteine sulfinic acid, the first (irreversible) step in taurine generation (p=0.002). In contrast, non-progressing AGMs had significantly lower levels than PTMs (p=0.004, p=0.002). At acute infection, taurine was the best predictor of rapid progression in PTMs (p=0.01). By setpoint, rapid-progressing PTMs had higher glycolysis-related metabolites like pyruvate (p<0.05). In contrast, AGMs experienced shifts to increased glutathione cycling metabolites by setpoint (all p<0.01). These were accompanied by increased acyl carnitines (all p<0.001), suggesting increased fatty acid oxidation (FAO). In PTMs, glutathione cycling metabolites were negatively associated with markers of disease progression, while taurine was correlated with glycolysis-associated cytokines

Cysteine utilization before and following infection predicted disease progression in nonhuman primates. The limited amino acid cysteine is critical for energy metabolism through either taurine or glutathione synthesis for glycolytic (pro-activating) or oxidative phosphorylation (pro-regulatory) immunometabolic responses, respectively. Glutathione is required for FAO (preceding oxidative phosphorylation) and neutralizes reactive oxygen species produced during ATP generation, preventing immune cell death. Defining cysteine metabolites' roles in immunometabolism will help uncover therapeutic strategies to improve immunological responses for PWH.

A Biomimetic Human Bruch's Membrane as a Potential Scaffold for Vision Restoration

Joy, Jincy¹; Flohr, Keevon²; Cargill, Casey¹; Lee, Daniel M.W.^{1,2}; Khandaker, Irena²; Rossi, Ethan A.^{1,2}; Chhablani, Jay²; Sahel, José-Alain^{2,5,6,7,8,9}; da Silva, Susana^{1,2,3}; Vande Geest, Jonathan P.^{1,2,4}

¹Department of Bioengineering, University of Pittsburgh

²Department of Ophthalmology, University of Pittsburgh

³Center for Neuroscience, University of Pittsburgh

⁴Department of Mechanical Engineering and Material Science, University of Pittsburgh

⁵Hôpital Fondation A. de Rothschild, Paris, France

⁶Hôpital National des Quinze-Vingts, Paris, France

⁷INSERM, General Directorate for Healthcare Organization, Clinical Investigation Center 1423, Paris, France

⁸Sorbonne Université, Paris, France

⁹Institut de la Vision, Paris, France

Purpose: A transplantable scaffold for retinal pigment epithelium (RPE) cell replacement therapy is a promising approach to restore vision, especially for macular degeneration. We present a novel and handleable protein nanofibrous scaffold, bioengineered to mimic the specific properties of the human Bruch's membrane (BM) to create a biomimetic substrate for human-induced pluripotent stem cell-derived RPE (hiRPE) cells.

Methods: Electrospinning was utilized to fabricate the biomimetic BM (B-BM) scaffold and match the trilayered architecture, topography and collagen-based composition of the human BM. hiRPE cells, were seeded (passage (P) 4; density: 1×10^5) on B-BM scaffold for 120 days (D). Live *in vitro* hiRPE behavior on B-BM was monitored, and cellular features at D120 were characterized using immunohistochemical staining, electron microscopy and morphometric analysis.

Results: The B-BM emulated the gradient porosity where the top layer resembled the 60-70 nm fiber diameter of the inner collagenous layer of the human BM. The B-BM promoted maturation of hiRPE cells and displayed cobblestone morphology and deep pigmentation. The polarized monolayer of hiRPE on B-BM (P4D92) expressed RPE-specific markers. We observed three distinct structural variants of basal infoldings, unprecedented on our B-BM scaffold. We identified cylindrical microvilli and two morphological variants of sheet-like microvilli, which were observed for the first time on our scaffold. The morphometric cell area of hiRPE on B-BM resembled the human retinal regions from the fovea to perifovea, constituting the macula.

Conclusion: The biomimetic features of B-BM promoted hiRPE features that resembled the human RPE, and especially, the sheet-like microvilli might facilitate visual improvement post-implantation. Remarkably, the macular resemblance accentuates its utilization for macular transplantation and as an *in vitro* model to understand the foveal adaptations.

Higher ADHD Symptom Awareness is Associated with Increased Positive Urgency Among Individuals with ADHD

Jusko, Morgan L.¹; Williard, Aiden¹; Pedersen, Sarah L.¹; Molina, Brooke S. G.¹; Kennedy, Traci M.¹

¹Department of Psychiatry, School of Medicine, University of Pittsburgh

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder associated with symptoms of inattention, hyperactivity, and impulsivity that can cause substantial impairment for emerging adults. According to executive functioning models, individuals with ADHD often have difficulties self-monitoring their symptoms and behavior. In turn, poor awareness of heightened in-the-moment symptoms could lead to a greater likelihood of impulsive action, both when in a positive (positive urgency) and a negative (negative urgency) mood. The current study examined associations of in-the-moment symptom awareness with negative and positive urgency. We predicted that moments of lower symptom awareness than usual would be related to higher impulsivity.

For 21 days, 36 emerging adults (ages 18-21) with rigorously diagnosed ADHD (69% female) completed five EMA prompts per day of their ADHD symptoms, awareness of symptoms, and impulsivity. Data were analyzed using multilevel regression modeling, adjusting for sex and student status.

Results revealed a significant between-person association of ADHD symptom awareness with positive urgency ($p < .01$), such that individuals with higher average symptom awareness across prompts tended to report greater positive urgency. There was no within-person effect ($p = .09$). Female participants reported lower positive urgency on average ($p < .01$). There were no significant findings for negative urgency.

In contrast to our hypothesis, *more* awareness of ADHD symptoms on average was associated with *higher* positive urgency. Future work should examine prospective associations of self-awareness with subsequent ratings of momentary impulsivity, as expected effects of lower awareness may unfold over a matter of hours. Objective measures of awareness are crucial to elucidating the role of this construct in impulsivity and resulting impairments.

Exploring the role of unfolded protein response as a target for treating *Aspergillus* keratitis

Kamath, Manali M.^{1,3}; Adams, Emily M.²; Wells, Becca L.¹; Lightfoot, Jorge D.¹; Fuller, Kevin K.^{1,2,3}

¹Department of Microbiology & Immunology, University of Oklahoma Health Sciences Center

²Department of Ophthalmology, University of Oklahoma Health Sciences Center

³Department of Ophthalmology, University of Pittsburgh School of Medicine

Purpose

We have demonstrated that genetic ablation of the *Aspergillus fumigatus* unfolded protein response (UPR) blocks the ability of the fungus to establish infection in a murine model of fungal keratitis. In this study, we explore the antifungal activity of a mammalian UPR inhibitor *in vitro* as well as the drug's ocular toxicity profile and therapeutic potential *in vivo* using ocular imaging techniques such as optical coherence tomography (OCT).

Methods

The antifungal properties of 4 μ 8C were tested *in vitro* on various *Aspergillus* species in a microbroth dilution assay and its specific action on *hacA* mRNA splicing by RT-PCR. Ocular toxicity post-treatment with 4 μ 8C topical drops three times/day was evaluated by H&E staining, slit-lamp imaging and corneal thickness measured by OCT. The antifungal activity of the drug was tested in murine model of fungal keratitis by topically administering 4 μ 8C/vehicle to infected eyes. Disease severity and fungal burden were evaluated at 72 hours post-inoculation using colony-forming units.

Results

The UPR inhibitor 4 μ 8C demonstrated dose-dependent fungicidal effect against *A. fumigatus*. The drug had minimal side effects on the integrity of the cornea rendering it safe for *in vivo* use. When used as treatment for infected eyes, 4 μ 8C significantly reduced the fungal burden compared to controls. Clinical severity was also reduced based on slit-lamp imaging. Treated eyes further retained normal corneal thickness, indicating absence of corneal edema/inflammation.

Conclusion

We demonstrate that the UPR pathway is crucial for the establishment of infection for *A. fumigatus* during keratitis. The mammalian Ire1 inhibitor, 4 μ 8C, significantly reduces fungal growth in a murine FK model, suggesting its potential for repurposing for treating *Aspergillus* keratitis.

3D Reconstruction of Myelin Ultrastructure in Postmortem Human Brain Tissue via AI-Assisted Segmentation

Kozel, Jenesis¹; Maier, Matthew M.^{1,2}; Durbin, Jamie³; Campos, Emily⁴; Glausier, Jill R.^{1,2}

¹Department of Psychiatry, University of Pittsburgh

²Center for Neuroscience, University of Pittsburgh

³Department of Psychiatry, University of Pittsburgh Medical Center, Western Psychiatric Hospital

⁴Department of Neuroscience, University of Pittsburgh

Higher-level cognitive processes, such as working memory, require tightly synchronized intra- and inter-cortical neural communication. To efficiently conduct electrical neural signals, axons are wrapped in a multi-layered lipid sheath termed myelin that provides insulation and energetic support. Defining features of myelin include its compact, intact layers, which prevents electrical signal leaking as it travels down the axon, and thickness of the myelin sheath itself, which decreases membrane capacitance and increases neuroconduction speed. If these morphological features are compromised, via split layers or reduced volume, neurotransmission may be disrupted.

Myelin is lipid-rich, which is optimal for imaging via focused-ion beam scanning electron microscopy (FIBSEM) in three-dimensions and at a nanometer resolution. Thus, the focus of this work is to characterize myelin morphology in postmortem human dorsolateral prefrontal cortex (DLPFC), a brain region critical for higher-level cognitive processes. Using a Helios 5 UC Dualbeam microscope (Thermo Fisher), volumetric data of 20 nm³ isotropic voxel size was captured from postmortem human DLPFC (42.6 x 25.4 x 11.3 mm, 567 Z-slices). Following volume acquisition, myelin was segmented and reconstructed using Amira software (Thermo Fisher). Traditional manual segmentation is often completed in a nearly slice-by-slice manner. To accelerate this process, deep learning models embedded in Amira were trained to recognize and segment myelin. This required initial manual segmentation of only 33 regions of interest and training that was completed in 47 minutes, producing an intersection over union score of 0.9863, a metric of high objection detection model accuracy. After human validation of the deep learning results and manual segmentation clean-up, quantitative measures of myelin morphology, including volume, and qualitative descriptions, such as layer integrity, were obtained. Thus, utilization of FIBSEM paired with AI-assisted segmentation enables accelerated characterization of myelin morphology, providing proxy measures of the state of neuroconduction in healthy and neuropsychiatric contexts.

Modeling Neural Responses to Prolonged Intracortical Microstimulation in the Mouse Visual Cortex

Kumar, Anal¹; Vazquez, Alberto L.²; Hooks, Bryan M.³; Gharbawie, Omar A.³; Gaunt, Robert A.⁴; Kozai, Takashi D. Y.⁵; Huang, Chengcheng¹

¹Department of Neuroscience, University of Pittsburgh

²Department of Radiology, University of Pittsburgh

³Department of Neurobiology, University of Pittsburgh

⁴Department of Physical Medicine and Rehabilitation, University of Pittsburgh

⁵Department of Bioengineering, University of Pittsburgh

Intracortical Microstimulation (ICMS) is a vital technique for providing sensory feedback in Brain-Machine Interfaces (BMIs) and sensory restoration. Despite decades of research, the precise neural mechanisms underlying ICMS remain poorly understood, particularly regarding the phenomenon of perceptual fading during prolonged stimulation.

To predict neural population responses evoked by ICMS, we developed phenomenological and spiking neural network (SNN) models to explain in-vivo calcium fluorescence data recorded from pyramidal neurons in the visual cortex of head-fixed, awake mice across a range of stimulation frequencies (10 Hz to 500 Hz) (Suematsu et al., 2024). Our phenomenological model achieved an exceptionally high goodness of fit ($R^2=0.98$) to the calcium fluorescence responses of single neurons. Virtual ablation of the different phenomenological components demonstrated the critical role of each mechanism: removing facilitation ($R^2=0.96$) eliminated the slow sensitization observed at low frequencies; omitting the metabolic resource depletion parameter ($R^2=0.90$) led to the absence of the rapid initial increase and subsequent desensitization; and removing divisive normalization ($R^2=0.68$) resulted in losing the inverse relationship between ICMS frequencies and neural calcium fluorescence responses. Expanding upon these single-cell insights, our SNN models the local circuit of a cortical layer and successfully captured the same inverse frequency-response relationship, alongside the transient increase and rapid desensitization.

Finally, we utilized an Evolutionary Algorithm (EA) to predict the optimal ICMS sequence required to generate the maximal neural response. Both the experimental data and the EA show that ~25 Hz stimulation generates this maximal response. In addition, we successfully used the EA to predict the stimulation sequences required to generate a specific target response (a 0.2 Hz sinusoid), demonstrating enhanced control over neural output. These findings offer a quantitative and mechanistic framework to overcome adaptation-induced perceptual fading, paving the way for optimized stimulation protocols, and more robust BMI applications.

Integrative structural and transcriptomic profiling reveals preservation of breast cancer genome architecture in patient-derived organoids

Kumar, Rahul¹; Shah, Osama¹; Schlegel, Brent T.¹; Hooda, Jagmohan¹; Brown, Daniel²; Chang, Hung-Ching³; Chen, Jian¹; Atkinson, Jennifer, M.¹; Mattila, Polly²; Fairfull, Liane¹; Tseng, George⁴; Bhargava, Rohit⁵; Oesterreich, Steffi¹; Lee, Adrian V.^{1,2}

¹Women's Cancer Research Center, UPMC Hillman Cancer Center and Magee-Women's Research Institute, University of Pittsburgh

²Institute for Precision Medicine, University of Pittsburgh

³Department of Biostatistics, University of Pittsburgh

⁴School of Public Health, University of Pittsburgh

⁵Department of Pathology, University of Pittsburgh

DNA structural variation (SV) is a prominent feature of breast cancer genomes. Some of these alterations arise through defective DNA repair, including homologous recombination deficiency (HRD), as well as genome remodeling mechanisms such as chromothripsis and breakage–fusion–bridge (BFB) cycles. To determine whether patient-derived organoids (PDOs) capture this complexity, we performed an integrative analysis of structural and transcriptional alterations across 27 breast tumors and 35 unmatched PDOs using optical genome mapping (OGM) and RNA sequencing.

OGM revealed widespread SV across both cohorts, with burdens ranging from 3–348 events in PDOs and 7–595 in tumors. Deletions were the dominant SV class in both groups, whereas PDOs were enriched for intrachromosomal translocations, and tumors showed more interchromosomal events. Breakpoint recurrence was limited, indicating that most rearrangements were sample-specific. Nevertheless, PDOs recapitulated the breast cancer SV architecture observed in tumors, including alterations involving driver genes such as CCND1, PPM1D, BRIP1, CDH1, FOXA1, and PIK3CA. The SV landscape was not identical between PDOs and tumors, consistent with preservation of tumor genome structure and model-associated selection.

HRD scores were broadly distributed in both cohorts, with a subset of samples showing genomic instability. HRD-high genomes displayed rearrangement patterns and copy-number disruption relative to HRD-low genomes. We identified 216 amplicons in PDOs and 131 in tumors, whereas only a small fraction showed evidence of BFB-associated mechanisms. Chromothripsis was also detected in both groups, highlighting genome remodeling in these samples.

Gene fusion analysis identified 1,147 DNA-level fusion candidates by OGM and 383 transcript-level fusion events by RNA sequencing, but only 37 were supported by both platforms. Expression-based prioritization refined these to 330 functional fusions in PDOs and 341 in tumors, indicating that only a restricted subset of genomic rearrangements yields detectable transcripts. Together, these findings establish PDOs as faithful yet dynamic models of breast cancer genome architecture.

A Single Arm Cuff for Non-invasive Monitoring of Cardiac Output, Arterial, and Venous Pressure

Kumar, Ravinder¹; Daher, Hadi¹; Jazini, Mahdi¹; Dhamotharan, Vishaal¹; Satheam, Abhijeet³; Longhitano, Yaroslava³; Abuelkasem, Ezeldeen³; Chandrasekhar, Anand¹; Pinsky, Michael⁴; Subramaniam, Kathirvel³; Planinsic, Raymond³; Hahn, Jin-Oh²; Shroff, Sanjeev¹; Howard-Quijano, Kimberly³; Mahajan, Aman³; Mukkamala, Rama^{1,3}

¹Department of Bioengineering, University of Pittsburgh

²Department of Mechanical Engineering, University of Maryland College Park

³Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh

⁴Department of Critical Care Medicine, University of Pittsburgh

Introduction: Hemodynamic monitoring of cardiac output (CO), venous pressure (VP), and blood pressure (BP) is central to perioperative care. However, comprehensive assessment typically requires multiple modalities that are invasive, operator dependent, or resource intensive. Automated arm cuffs are routinely used for non-invasive BP measurement and generate pressure oscillations reflecting vascular volume changes. These oscillations peak near mean arterial pressure (MAP) due to the arterial pressure–volume relationship, and analogous low-pressure oscillatory features may appear with elevated VP. We hypothesized that a standard automated arm cuff could noninvasively estimate CO and MAP and detect elevated VP using cuff-derived pulsatile signals.

Methods: A custom automated arm cuff device was developed to record cuff pressure during servo-controlled linear deflation. A standard oscillometric protocol was used for CO estimation, and a modified low-pressure protocol with inflation limited to 50 mmHg and slower deflation was used for venous assessment. An integrated airflow sensor enabled direct measurement of air volume changes, allowing derivation of the arm cuff pressure–volume relationship. Under IRB approval, measurements were collected during hemodynamic interventions in liver transplant and cardiac surgeries. Reference signals included invasive arterial pressure, central venous pressure (CVP), and pulmonary artery thermodilution CO. CO was estimated from HR, maximal oscillation amplitude, arm–cuff compliance, and a body-to-arm surface area scaling factor. MAP was estimated using the peak of a “volume oscillogram,” defined as compliance multiplied by oscillation amplitude. Low-pressure oscillation peaks were analyzed for classification of CVP > 12 mmHg.

Results: Cuff-estimated CO showed agreement with reference values ($r = 0.60$; $N = 34$). The proposed MAP method improved correlation with invasive MAP from 0.79 to 0.86 and reduced bias and precision to -0.83 mmHg and 6.5 mmHg. In another cohort ($N = 8$), VP detection achieved 68% sensitivity and 89% specificity.

Conclusion: These findings suggest that CO, MAP, and VP may be assessed noninvasively using a single automated arm cuff.

Research/Grant Support: This work was supported by the National Institutes of Health under Grant HL163691.

Optimized dried plasma spot workflow increases biomarker detectability for Alzheimer's disease research

Lee, You-Rim¹; Zeng, Xuemei¹; Farinas, Marissa F.¹; Pierce, Madelyn K.¹; Nicholson, Nya¹; Cohen, Ann D.²; Lopez, Oscar L.³; Karikari, Thomas K.¹

¹**Department of Psychiatry, University of Pittsburgh**

²**University of Pittsburgh Alzheimer's Disease Research Center (ADRC)**

³**University of Pittsburgh School of Medicine, Pittsburgh, PA, USA**

Introduction: Dried plasma spots (DPS) offer advantages over traditional venous blood collection for widescale Alzheimer's disease (AD) biomarker research, including self-collection, simplified transport, and reduced infrastructure requirements. However, no study has systematically optimized the DPS workflow considering real-world challenges such as sample stability, blood volume variability, and limited detection sensitivity from low sample input. We aimed to address these challenges to enable broader applications of DPS.

Methods: Matched plasma and DPS samples were prepared from the University of Pittsburgh ADRC participants. For the stability assessment, DPS cards were stored at room temperature for 0, 1, 3, and 7 days. For blood volume optimization, 10–100 μ L of whole blood was applied onto Telimmune DPS cards. To improve sensitivity, we evaluated the use of SpeedVac for sample concentration. 127 neurodegenerative biomarkers were analyzed using Nucleic Acid-Linked Immuno-Sandwich Assay (NULISA)seq CNS Disease Panel.

Results: In the stability experiment, detectability remained stable (84–87%) across Day 0–7. Recovery rate analysis showed 84% of targets had acceptable recovery from plasma, and 88% maintained stable retention (85–115%) through Day 7. Among 8 classical AD biomarkers, 6 remained stable: A β 42, GFAP, NEFL, pTau-181, pTau-217, and pTau-231. In the blood volume experiment, 70 μ L showed optimal absorption, the lowest CV in total protein analysis, and highest correlation with EDTA plasma ($\rho=0.53$, $p<0.0001$). SpeedVac drying significantly improved biomarker detectability from 71% (using the manufacturer's standard protocol) to 85%, with notable improvements for GFAP (0→100%), NEFL (50→100%), and pTau-217 (0→70%), while correlation with plasma was maintained.

Conclusion: Our workflow leads to increased sensitivity of biomarker levels extracted by DPS, enabling feasibility for everyday use. Importantly, the introduction of a novel SpeedVac drying step vastly improves sample detectability compared to standard protocols. This work establishes key preanalytical parameters needed for evidence-based application of DPS in AD research.

Dual Inhibition of iNOS and COX2 Enhances Antitumor Immunity and Improves Response to Immune Checkpoint Blockade in Hepatocellular Carcinoma

Li, Yuzhen¹; Wang, Ronghua¹; Wu, Qianru¹; Yazdani, Hamza¹; Chen, Tianmeng¹; He, Zhengyi^{1,3}; Tohme, Samer¹; Billiar, Timothy¹; Kaltenmeier, Christof^{1,2}

¹Department of Surgery, University of Pittsburgh Medical Center

²MedStar Georgetown University Hospital, Washington, DC

³School of Medicine, Tsinghua University, Beijing

Background: Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality, often presenting at advanced stages. Although immune checkpoint blockade (ICB) improves outcomes in some patients, efficacy is limited by an immunosuppressive tumor microenvironment. Inducible nitric oxide synthase (iNOS/NOS2) and cyclooxygenase-2 (COX2) are inflammation-associated enzymes that regulate immune responses and are frequently upregulated in cancer. We hypothesized that iNOS and COX2 suppress antitumor immunity and that their inhibition enhances response to ICB in HCC.

Methods: Tumor tissues from 75 HCC patients were analyzed by immunohistochemistry for NOS2, COX2, and CD8. Overall survival was assessed by Kaplan–Meier analysis. In an orthotopic murine HCC model (RIL-175), mice received vehicle, NOS2 inhibitor (1400W), COX2 inhibitor (celecoxib), anti–PD-L1, or combination therapies. Tumors were analyzed after two weeks using flow cytometry, spatial imaging, cytokine assays, and CITE-seq. In vitro, murine splenocytes were treated with PGE2 or nitric oxide donor (DETA-NONOate), and CD226/TIGIT expression was assessed.

Results: High NOS2 expression was associated with reduced survival (HR 3.65, $p=0.0011$), with worse outcomes in NOS2/COX2 co-expression (HR 9.31, $p<0.0001$). Dual inhibition reduced tumor burden and increased CD8⁺ and CD4⁺ T-cell infiltration, B-cell recruitment, and circulating IL-2 and IFN- γ . Triple therapy with anti–PD-L1 further improved tumor control and survival. Single-cell analysis showed enhanced CD8⁺ T-cell activation with increased CD226 and decreased TIGIT expression. In vitro, nitric oxide and PGE2 impaired CD8⁺ T-cell function by reducing CD226 and increasing TIGIT.

Conclusions: iNOS and COX2 drive an immunosuppressive program in HCC that limits T-cell–mediated antitumor immunity. Dual targeting enhances immune activation and improves response to checkpoint blockade, representing a promising therapeutic strategy.

Feasibility of Non-Expert Annotation for Building Labeled Breast Dense Tissue Mask Datasets in Digital Breast Tomosynthesis

Liu, Yen-Tung¹; de Méro Omena, Luana²; Muniz, Guilherme²; Mustafaev, Tamerlan¹; Nishikawa, Robert M.¹; Barufaldi, Bruno³; Lee, Juhun¹

¹Department of Radiology, University of Pittsburgh

²Center of Informatics, Federal University of Paraíba, Paraíba, Brazil

³Department of Radiology, University of Pennsylvania

Introduction

Obtaining labeled medical imaging datasets is difficult due to the high cost of expert annotation. We previously developed a dense tissue mask generation tool that extrapolates manual segmentation from the center slice of a Digital Breast Tomosynthesis (DBT) volume. We aim to test if non-experts can use this tool to generate labeled datasets that comply with clinical standards.

Hypothesis

Non-experts can provide clinically acceptable fibroglandular tissue mask compared to experts.

Methods

Two radiologists (experts) and three researchers (non-experts) independently segmented fibroglandular tissue on the center slice of 60 DBT images (30 patients, bilateral CC views) from the publicly available DBTex database. Non-experts received a brief training session on fibroglandular tissue regions demonstrated by one expert. Percent density (PD), defined as fibroglandular tissue divided by breast area, served as the comparison metric. Agreement was assessed using bootstrapping (10,000 resamples) to quantify the mean difference in PD, and Bland-Altman analysis to evaluate the difference relative to mean PD.

Results

A statistically significant positive bias of 9.94% PD (95% CI: 6.65% to 13.14%) was observed in non-expert estimates relative to expert PDs, indicating non-experts consistently overestimated PD. The limits of agreement spanned from -8.29% to 28.18%. The overestimation was most evident in low PD (fatty) cases, where the bias was high despite relatively low variance, likely due to difficulty removing non-fibroglandular tissue like vessels. As PD increased, the bias decreased (closer to 0), suggesting that non-experts' estimates aligned more closely with experts in dense cases. This improved accuracy, despite greater variability, resulted in most denser cases (PD $\geq$ 40%) falling within the clinically accepted threshold of 10%.

Conclusion

Although there was 9.94% mean overestimation by non-experts compared to experts, it remains within the generally considered clinical acceptance threshold of 10%. Higher agreement was observed for denser cases, showing the potential of our tool for non-expert use.

The Combined Roles of Vesicular Release and Dopamine Reverse Transport on the Psychostimulant Properties of Amphetamine

Mabry, Samuel¹

Department of Psychiatry, University of Pittsburgh¹

Amphetamine (AMPH), a psychostimulant that is commonly prescribed for the treatment of neuropsychiatric disorders, has a high liability for abuse. The psychostimulant properties of AMPH are primarily associated with its ability to increase dopamine (DA) neurotransmission by eliciting a reversal of the DA transporter (DAT). This phenomenon, which is called DA efflux, occurs in response to AMPH and causes the DAT to operate in reverse, allowing DA to flow from the cytosol to the synapse. However, how AMPH elicits accumulation of cytosolic DA that permits this DA efflux remains unclear. Importantly, when AMPH is uptaken by DAT, it causes a depolarization of DA neurons. This depolarization can initiate vesicular DA release, thus giving AMPH a potential secondary mechanism for increasing DA neurotransmission beyond just DA efflux. We propose two-part mechanism for AMPH-induced DA release. The initial phase of DA release in response to AMPH is caused by DAT-mediated depolarization due to uptake of AMPH, which enhances vesicular DA release. This subsequently primes the DA neurons for DA efflux by increasing levels of cytoplasmic DA upon reuptake through DAT. Once DAT is primed for efflux and cytoplasmic DA levels have sufficiently increased, DA efflux will occur, driving the prolonged psychostimulant effects of AMPH. Using *Drosophila melanogaster* as our animal model, we probed the behavioral effects of AMPH and used genetic and pharmacological approaches to block vesicular release and DA efflux, respectively. Utilizing a combination of behavior, biosensors, and live imaging, we demonstrate that both vesicular release and DA efflux are critical to the psychostimulant properties of AMPH. Furthermore, vesicular release is critical for the DA accumulation that precedes and permits the occurrence of DA efflux. Together, this work demonstrates a new mechanism for AMPH where both vesicular release and DA efflux are critical for the psychostimulant properties of the drug.

Resolving differential vascular graft remodeling using longitudinal multiphoton tracking in a 3D culture platform

Maestas Jr., David¹; Murphy, Trin¹; Martinet, Katarina¹; Moyston, Tracey¹; Min, Leon^{1,2}; Behrangzade, Ali¹; Pemberton, Brock¹; Joy, Jincy¹; Ye, Sang-Ho¹; Hussey, George^{3,4}; Azhar, Mohamad^{5,6}; Wagner, William^{1,7,8}; Vande Geest, Jonathan^{1,9}

¹Department of Bioengineering, University of Pittsburgh

²Department of Medicine, University of Pittsburgh

³Department of Surgery, University of Pittsburgh

⁴McGowan Institute of Regenerative Medicine, University of Pittsburgh

⁵Department of Cell Biology & Anatomy, School of Medicine, University of South Carolina

⁶Department of Biomedical Engineering Program, University of South Carolina

⁷Department of Chemical and Petroleum Engineering, University of Pittsburgh

⁸Department of Mechanical Engineering and Materials Science, University of Pittsburgh

⁹Vascular Medicine Institute, University of Pittsburgh

The long-term performance of tissue-engineered scaffolds, particularly small-diameter vascular grafts, is shaped by remodeling events at the tissue-graft interface, yet these processes remain difficult to resolve longitudinally and at microstructural resolution in conventional implantation models. Here we develop an organotypic artery-graft model that preserves cylindrical vessel geometry and enables non-destructive label-free multiphoton monitoring of interface remodeling. Using second harmonic generation and two-photon excited fluorescence, we capture evolving fibrillar collagen architecture and cellularization over time, demonstrate compatibility with multiple biomaterial classes, and show integration with rat and mouse explants, live-cell dyes, and fluorescent reporter tissues. The platform resolved distinct remodeling responses to transforming growth factor- β isoforms (TGF- β 1, - β 2, and - β 3), with differential shifts in collagen-fiber distributions, accompanied by changes in matrix-remodeling and contractile gene expression. Across two graft designs, culture-derived remodeling phenotypes, collagen fiber distributions, and initial trajectories agreed with those observed in long-term 6-month interpositional explants. Together, these results establish an accessible intermediate platform for interrogating artery-graft remodeling, tracking these trajectories, and prioritizing graft designs through interface-resolved outcomes before and alongside animal implantation studies.

Extracellular Matrix hydrogel promotes the long-term growth and function of thyroid organoids.

Marin, Paula^{1,2}; Johnson, Scott¹; Mathews, Lisa^{3,4}; Naaz, Afsana^{3,4}; Turnquist, Heth^{3,4}; Badylak, Stephen F.^{1, 2}; Hussey, George^{1,2,5}

¹McGowan Institute for Regenerative Medicine, University of Pittsburgh

²Department of Surgery, University of Pittsburgh

³Department of Immunology, University of Pittsburgh

⁴Thomas E. Starzl Transplantation Institute, University of Pittsburgh

⁵Department of Bioengineering, University of Pittsburgh

The function of thyroid hormones includes promoting tissue growth, neuronal development, reproduction, and regulation of energy metabolism. Thyroid disorders, such as hypothyroidism and hyperthyroidism, are prevalent and can have profound effects upon health. Current treatments for thyroid disorders include hormone replacement therapy and thyroid transplantation, both of which have limitations such as control of dose and immune rejection. The development of implantable, cell-based devices that produce and release the necessary and appropriate dose of a hormone on demand represents an exciting alternative approach to treating thyroid disorders. However, cell-based sensing and hormone delivery systems rely on appropriate biomaterials to promote and maintain the growth and function of transplanted cells. The objective of the present study was to evaluate the ability of an ECM hydrogel composed of urinary bladder matrix (UBM) to sustain long-term organoid growth and function of transplantable thyroid cells. iPSC-derived thyroid cells were cultured for up to 90 days within a UBM hydrogel. Viability of cells was assessed by flow cytometry using fixable viability dyes. Cell organization and lumen formation were evaluated by phase contrast microscopy and Hematoxylin/Eosin staining. Thyroglobulin expression was evaluated by RT-qPCR and Thyroxine (T4) released by ELISA. A murine in vivo model was used to assess vascularization after eight weeks. In vitro, cells cultured within the ECM hydrogel formed organoids exhibiting greater than 88% viability for up to 90 days, the presence of colloid within the lumen, and elevated thyroglobulin and hormone secretion. In vivo, the ECM hydrogels promoted host vasculature at a density statistically similar to native tissue. In summary, the results showed that ECM hydrogel promotes a vascularized microenvironment that supports the formation, viability and function of 3D thyroid organoids. These studies represent a proof-of-concept for future therapeutic development and incorporation of ECM hydrogels into cell-based devices for restoring dysregulated endocrine function.

Loss of actin-binding protein Profilin1 drives a pro-inflammatory phenotype in kidney endothelial cells in a Tripartite Motif 24 (TRIM24)-dependent manner

Maurya, Sudeep Kumar¹; Tuan, Grace¹; Shetty, Ananya²; Gau, David^{1,3}; Roy, Partha^{1,3}

¹Department of Bioengineering, University of Pittsburgh

²Department of Molecular Biology, University of Pittsburgh

³Department of Pathology, University of Pittsburgh

Background. Transcriptional upregulation of actin-binding protein Profilin-1 (Pfn1) is associated with adverse disease outcome in human clear cell renal cell carcinoma (ccRCC). Our previous studies found that Pfn1 is predominantly upregulated in tumor-associated vascular endothelial cells (ECs). Mouse model studies and clinical correlative analyses further established a direct causal relationship between EC Pfn1 dysregulation, tumor immune microenvironment modulation, and disease progression in RCC. The goal of this study is to gain mechanistic insights into how endothelial Pfn1's action negatively regulates T cell trafficking into RCC tumors.

Results. We performed studies utilizing an hTERT-immortalized murine kidney EC line harboring floxed Pfn1 alleles to first demonstrate that genetic loss of Pfn1 function in kidney ECs promotes chemotactic migration of both CD4 and CD8 T cells via transcriptionally upregulating T-cell recruiting chemokines (CCL5 and CXCL10) through increased activation of pro-inflammatory NFkb and STAT1/Interferon signaling. By upstream regulator analyses of transcriptomic data of murine RCC tumor samples as well as cultured ECs, we further identified tripartite-motif containing-24 (TRIM24), a dual-function ubiquitin-ligase/transcriptional modulator, to be robustly functionally inhibited in the absence of Pfn1 expression in ECs. Triggering functional loss of TRIM24 by either a selective bifunctional degrader or a small molecule inhibitor phenocopies the pro-inflammatory signatures of Pfn1-deficient ECs. We further established two divergent TRIM24-regulated downstream signaling axes, namely mitochondrial ROS-driven activation of p38MAPK/NFkb and retinoic acid receptor (RAR)-dependent STAT1 modulation, that link EC-Pfn1's loss to stimulation of CCL5/CXCL10-driven chemotactic migration of T cells.

Conclusions. These findings suggest that TRIM24 plays a central role in endothelial Pfn1-dependent control of chemical milieu for T-cell trafficking, and therefore Pfn1-TRIM24 axis may represent a novel therapeutic target for modulating inflammatory responses in vascular tissues and RCC TME.

TGFBI modulates fibroblast phenotype inhibiting CD8⁺ T cells and NK Cell tumor Infiltration

Mazumder, Md Habibul Hasan^{1,2}; Gomes-Jourdan, Noemie^{1,2}; Kean, Tia^{1,2}; Zhang, Liana^{1,3}; Cascio, Sandra^{1,2} *; Buckanovich, Ronald^{1,2} *

¹Magee Women's Research Institute

²Department of Obstetrics Gynecology and Reproductive Sciences, University of Pittsburgh

³Department of Applied Mathematics, Ningbo University

*Co-senior authors

TGFBI (Transforming Growth Factor Beta-Induced), a stromal factor highly expressed by cancer-associated mesenchymal stem cells (CA-MSC), correlates with immunotherapy resistance. To investigate the immunoregulatory role of TGFBI, C57BL/6 wild-type (WT) and *Tgfbi* knockout (KO) mice were injected with immune 'hot' UPK-10 OvCa cells. KO mice showed 50% reduction in tumor growth compared to WT mice. Flow cytometric and multispectral imaging analyses showed significant increased tumor infiltration of CD8⁺ T and NK cells and reduced CD206⁺ macrophages in KO mice.

Adipose-derived MSCs isolated from WT or *Tgfbi*-KO mice were co-injected with UPK-10 cells (1:1 ratio) in WT mice and KO mice to investigate specific effect of MSC-derived *tgfb*i in immune regulation. Lack of *Tgfbi* in MSC promoted tumor infiltration of cytotoxic CD8 T and NK cells compared to control, suggesting that MSC-derived *Tgfbi* may directly drive immune cell exclusion. Adhesion and migration assays indicated that CD8⁺ T and NK cells adhere more to KO MSC compared to WT MSC. Additionally, qPCR revealed that fibroblast infiltrating KO-tumors exhibit an increased signature of anti-tumorigenic inflammatory fibroblasts (*IL-6*, *CXCL-14*)-and reduced pro-tumorigenic myofibroblast markers (*Col1a1*, *α -SMA*), suggesting a broader role for TGFBI in stromal regulation. Spatial transcriptomic analysis of human OvCa samples demonstrated that TGFBI expression spatially correlated with integrin β 1, and co-immunoprecipitation confirmed a direct interaction between TGFBI and integrin β 1.

Collectively, these findings demonstrate that TGFBI plays a critical role in promoting immune cell exclusion within the ovarian tumor microenvironment and highlights TGFBI as a promising therapeutic target for OvCa.

PPAR α Modulation by Doxorubicin Underlies Sex Specific Cardiotoxicity

Melcher, Amber V.¹; George, Sharon A.^{1,2}

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh

²Department of Bioengineering, University of Pittsburgh

The heart relies on fatty acids (FA) for energy. Doxorubicin (DOX) is a chemotherapeutic agent known to cause cardiotoxicity and dysregulation of FA metabolism. PPAR α (Peroxisome Proliferator Activated Receptor alpha) is essential for cardiac FA utilization. Preliminary data indicates activation of PPAR α is higher in females compared to males with DOX and might lead to better cardiac-outcomes in females. We tested the hypothesis that loss of PPAR α would eliminate sex-specific cardioprotection in females during DOX treatment. Echocardiography and quadruple-parametric-optical-mapping was used to simultaneously image transmembrane-potential, calcium-handling, NADH, and FAD intensity in male and female mice. Wild-type (WT), WT+DOX, PPAR α global knock-out (KO), KO+DOX, DOX were intraperitoneally injected 5-days before measurements (20mg/kg). In males, WT+DOX had reduced ejection fraction (58 ± 6.01 , 66 ± 8.42) while females were reduced only in the KO+DOX group (56.07 ± 7.11 , 71 ± 4.86). Thus, DOX induced mechanical dysfunction in WT males but not females. With optical mapping, reporting as a percent change, WT+DOX males had increased voltage (V_m) rise-time (RT), action potential duration (APD), and calcium (Ca)RT (45 ± 2.7 , 27 ± 5.4 , 49 ± 14.2) compared to WT, indicating increased risk of arrhythmias with DOX. KO males had an increase in NADH and FAD intensity (3.4 ± 1.3 , 0.79 ± 0.45) compared to WT suggesting metabolic impairment. KO+DOX males had no changes compared to WT. In females, WT+DOX had arrhythmogenic slowing of transverse conduction velocity (CV_T , -10 ± 4.8) and reduced CaT (-54 ± 20) compared to WT. KO improved electrophysiology in females by increasing CV_T (36 ± 8.9 , CV_L 21 ± 11), reducing CaRT (-23 ± 6.1), CaT (-25 ± 19) and V_m -Ca Delay (-28 ± 17) compared to WT. KO+DOX reduced CaT and V_m -Ca delay (-43 ± 13 , -10 ± 20) compared to WT, returning these parameters to baseline. Overall, when PPAR α was genetically ablated, DOX induced sex-specific differences in cardiac mechanics and electrophysiology were abolished, and female sex specific cardioprotection was lost. This indicates that PPAR α plays a critical role in cardioprotection in DOX treated females.

Differential tau-PET load predominance along a hierarchical trajectory determines clinical phenotypes in Alzheimer's disease

Mroué, Rayan¹; Povala, Guilherme¹; Bellaver, Bruna¹; Ferreira, Pamela Lukasewicz¹; Bauer-Negrini, Guilherme¹; Lussier, Firoza Z.¹; Amaral, Livia¹; Scop Medeiros, Marina¹; Ruppert, Emma¹; da Rocha, Andreia Silva¹; Rodrigues, Matheus Scarpato¹; Oliveira, Markley Silva¹; Soares, Carolina¹; Leffa, Douglas Teixeira¹; Saha, Pampa¹; Masdeu, Joseph²; Soleimani-Meigooni, David³; Fortea, Juan⁴; Lowe, Val⁵; Oh, Hwamee⁶; Pascual, Belen²; Gordon, Brian A.⁷; Rosa-Neto, Pedro⁸; Baker, Suzanne⁹; Pascoal, Tharick A.¹

¹Department of Psychiatry, University of Pittsburgh; ²Department of Neurology, Houston Methodist Research Institute; ³University of California San Francisco; ⁴Department of Neurology, Hospital de la Santa Creu i Sant Pau; ⁵Department of Radiology, Mayo Clinic; ⁶Department of Psychiatry and Human Behavior, Brown University; ⁷Department of Radiology, Washington University in St. Louis; ⁸Translational Neuroimaging Laboratory, McGill University Research Centre for Studies in Aging, Douglas Research Institute; ⁹Lawrence Berkeley National Laboratory

Tau pathology spreads progressively in Alzheimer's disease, but heterogeneity in its spatial distribution may contribute to differences in clinical presentation. We investigated patterns of tau deposition across disease progression and their cognitive correlates.

We studied 647 individuals from the HEAD study (57% cognitively unimpaired, 43% amnesic cognitively impaired) who underwent head-to-head tau-PET with Flortaucipir and MK6240. For each tracer, we extracted median SUVRs from the entorhinal cortex and seven large-scale brain networks: limbic, visual (Vis), default mode (Default), dorsal attention (DorsAttn), frontoparietal, somatomotor (SomMot), and salience/ventral attention (SalVentAttn). Event-based modeling (EBM) was fit separately for each tracer to estimate a canonical sequence of regional tau-PET positivity using a reference group. Within each participant, EBM-positive regions were ranked by SUVR to identify peak patterns of deposition. Stage-standardized tau-PET subtype images and voxel-wise contrasts were generated for each pattern and tracer. Cognition was assessed using linear regression models for MoCA and domain-specific composites.

Across tracers, EBM revealed a hierarchical sequence consistent with Braak-like stages: tau emerged in the entorhinal cortex, spread to the limbic network, and subsequently involved the remaining networks. Positional variance diagrams demonstrated diagonal dominance, indicating consistent ordering for both tracers. After regional positivity, tau accumulation was non-uniform across individuals. Within-participant rankings identified three dominant peak patterns: Vis, Default, and DorsAttn. Voxel-wise contrasts showed clear spatial differences in tau-PET uptake for both tracers. Across tracers, the Vis pattern showed worse MoCA and memory scores than DorsAttn, while DorsAttn showed worse executive function than Vis. The Vis and DorsAttn patterns also showed lower visuospatial scores than Default, whereas Default showed worse memory than DorsAttn.

These findings support a model in which tau pathology advances hierarchically in a Braak-like manner yet shows preferential accumulation within specific large-scale networks. This network-based concentration of tau may contribute to distinct clinical phenotypes in Alzheimer's disease.

Live-attenuated Rift Valley fever virus vaccination leads to durable, protective immunity in mice

Mueller Brown, Karina^{1,2}; Kindsvogel, Angel M.^{1,2}; Freeman, Tracey L.^{1,2}; Nathan E.^{1,2}; Wilson, Lindsay R.^{1,2}; Divens, Ashley M.^{1,2}; Xu, Lingqing^{1,2}; Barbeau, Dominique J.^{1,2}; Bird, Brian H.³; McElroy, Anita K.^{1,2}

¹Division of Pediatric Infectious Diseases, Department of Pediatrics, University of Pittsburgh

²Center for Vaccine Research, University of Pittsburgh

³One Health Institute, School of Veterinary Medicine, University of California

Rift Valley fever virus (RVFV) is a zoonotic arbovirus endemic to Africa and the Arabian Peninsula that can cause severe disease in humans and livestock. There are currently no licensed vaccines for human use, but several candidates are at the clinical trial stage including DDVaxTM, a preparation of live-attenuated RVFV lacking the nonstructural proteins NSs and NSm (Δ NSs Δ NSm). Multiple pre-clinical studies have shown that Δ NSs Δ NSm is safe and immunogenic, but the longevity of that immunity is unknown. Thus, a pilot study to examine the duration of humoral and cellular immunity following a single, low-dose Δ NSs Δ NSm vaccination in mouse models of RVFV hepatitis (C57BL/6) and encephalitis (CC057/Unc) over a period of 6 months was carried out. Humoral and cellular immunity from vaccination decreased over time but were comparable between mouse strains. Importantly, vaccination provided substantial protection from wild type (WT) RVFV challenge 6 months post vaccination. Based on these findings, a subsequent larger-scale study investigated the additional features of sex and dose in immunogenicity and protection provided by Δ NSs Δ NSm in the RVFV hepatitis model using C57BL/6 mice. Humoral immunity in the form of RVFV-specific neutralizing and binding antibodies decreased over time but remained detectable at the end of the study. RVFV-specific CD4⁺ T cells also decreased by 6 months post-vaccination. In contrast, virus-specific polyfunctional CD8⁺ T cells persisted at comparable levels throughout the study. Lastly, vaccinated animals were protected against lethal Rift Valley Fever (RVF) disease 6 months post-vaccination independent of dose or sex. Taken together, this work demonstrates that a single low-dose of Δ NSs Δ NSm vaccine elicits a durable humoral and cellular immune response that persists over months and protects mice against lethal RVF disease.

The gut microbiota programs ovarian immune homeostasis to prevent pathological inflammation and fibrosis.

Munyoki, Sarah K.¹⁻³; Goff, Julie P.¹⁻³; Sinar, Hayden¹⁻³; Turnquist, Hēth⁴; Jašarević, Eldin¹⁻³

¹Department of Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh

²Department of Computational and Systems Biology, University of Pittsburgh

³Magee-Womens Research Institute

⁴Thomas E. Starzl Transplantation Institute, University of Pittsburgh School of Medicine

The ovary undergoes cyclic tissue remodeling to support ovulation, follicle atresia, and corpus luteum regression. With age, this immune-dependent process becomes dysregulated, transitioning from efficient tissue repair to chronic inflammation and fibrosis. We discovered that gut microbiota-derived short-chain fatty acids (SCFAs) are critical in preventing pathological inflammation and fibrosis in the mouse ovary. SCFA depletion resulted in inflamed, fibrotic ovaries, diminished ovarian reserve, and reduced fertility, mirroring hallmarks of ovarian aging. These ovaries showed significant upregulation of the cytokine interleukin-33 (*Il33*). Restoring SCFA levels rescued the ovarian aging phenotype and *Il33* expression.

IL-33 regulates type 2 immune responses, including tissue remodeling, by activating group 2 innate lymphoid cells (ILC2s) that express its receptor, ST2. When dysregulated, IL-33 contributes to pathological inflammation and severe tissue damage. Single-cell RNA sequencing of CD45⁺ ovarian immune cells revealed that ILC2s are the IL-33-responsive cells and exclusively express *St2*, *Il5*, and *Il13*. These ILC2s were threefold more abundant in SCFA-depleted ovaries. Ovaries from global IL-33 knockout mice displayed increased primordial follicles, reduced fibrosis, and downregulated IL-33 signaling, and ECM remodeling genes compared to wild-type controls. ST2⁺ peripheral regulatory T cells (Tregs) homed to the ovary in response to increased *Il33* expression, suggesting they regulate IL-33-ILC2-driven inflammation. The immunosuppressive function of Tregs is impaired in the absence of SCFAs. These findings establish that microbiota-derived SCFAs program ovarian immune homeostasis by restraining the IL-33-ILC2 axis, and that disruption of this pathway accelerates ovarian aging.

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

Ashna, Nagpal^{1,2}; Schaich, Matthew A.^{1,2}; Weaver, Tyler M.³; Roginskaya, Vera²; Freudenthal, Bret D.³; Tomkinson, Alan E.⁴; Van Houten, Bennett^{1,2}

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh

²UPMC-Hillman Cancer Center, School of Medicine, University of Pittsburgh

³University of Kansas Medical Center

⁴University of New Mexico Comprehensive Cancer Center and the Departments of Internal Medicine, and Molecular Genetics & Microbiology, University of New Mexico Health Sciences Center

End joining process of DNA single strand breaks (SSB) by ligase enzymes are 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 (LIG3 α) during base excision repair (BER). To measure the dynamics of LIG3 α -XRCC1 interactions with SSB in duplex lambda DNA or a single 601 nucleosome core particle (NCP), we used a real time single-molecule approach to visualize DNA binding events of Halo-tagged LIG3 α with XRCC1-YFP from nuclear extracts. On duplex DNA, LIG3 α and XRCC1 showed strong binding affinity with ligatable nicks, with lifetimes of 5.4 s and 6.9 s respectively, and 17.3% of events being co-localized. The loss of 5'P decreased the dwell times for both LIG3 α & XRCC1 by ~1.5 fold shorter when treated with CIP, concluding that 5' P is important for LIG3 α binding. Domain analysis suggests that the unique N-terminal zinc finger (ZnF) of Lig3 α acts as an autoinhibitory domain for long-lived stable interactions and had a strong effect on the binding affinities. By deleting the ZnF domain of LIG3 α or just expressing ZnF-BRCT domain with XRCC1 construct made the lifetimes longer. LIG3 α and XRCC1 were found to bind to an undamaged 601 NCP with lifetimes of 68.0 s and 33.1 s, respectively, and colocalization frequency was 35.2%. Deletion analysis indicated that the N-terminal ZnF 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. These single molecule studies have allowed us to uncover and important binding pocket of LIG3 α binding to an undamaged 601 NCP.

PARP1-Dependent Rod Photoreceptor Death in an Inducible Zebrafish Model of Retinitis Pigmentosa

Nwagbo, Uzoamaka¹; Voight, Molly¹; Wang, Beichen¹; Zhang, Liyun¹

¹Department of Ophthalmology, UPMC Vision Institute, University of Pittsburgh

Introduction: Retinitis pigmentosa (RP) is one common form of inherited retinal degenerative disorders, characterized by progressive rod photoreceptor loss and eventual blindness, with no effective treatment for most patients. Although thousands of mutations have been linked to RP, the mechanism of rod cell death remains unclear. While apoptosis and necroptosis have been thought to be the primary pathway, recent evidence highlights parthanatos as a key mechanism, also implicated in major neurological diseases and mammalian RP models. Previously, we generated a controlled, rod-specific, inducible zebrafish RP model to mimic RP key phenotypes. The aim of this study is to identify the major cell death pathway in this model using CRISPR/Cas9 gene targeting.

Methods: Zebrafish *Tg(rho: YFP-NTR2.0)* expressing YFP and nitroreductase (NTR) in rod cells were used. NTR hydrolyzes the prodrug metronidazole (MTZ) into a DNA-damage agent, selectively ablating rods. A concentration of 12.5 μ M was optimized to induce rod cell loss within two days. CRISPR/Cas9 was used to disrupt key genes in each cell death pathway: *parp1* (parthanatos), *casp3a/3b* (apoptosis), and *ripk1l* (necroptosis). Injected embryos were raised and exposed to MTZ from 5 to 6 days post-fertilization (dpf). Surviving rods were quantified using ARQive assays based on rod-YFP expression, and gene knockdown was confirmed by qPCR. Each experiment was conducted with at least three biological replicates.

Results: Compared to controls, *parp1* disruption showed significant increase in rod survival, suggesting parthanatos as the major rod death mechanism. *casp3a/3b* disruption displayed only a mild protective effect, while *ripk1l* disruption had no effect. qPCR verified successful knockdown of all targeted genes.

Conclusion: Rod cell death mechanism in this inducible RP model closely resembles mammalian RP models, with PARP-dependent cell death playing the dominant role. This validates the zebrafish inducible RP model as a reliable and disease-relevant platform for studying RP mechanisms and evaluating potential therapies.

Uncovering the Mechanisms Behind Meiosis-Dysfunction Driven Somatic Ageing

Osman, A. Guled^{1*}; Burton, Nick²; Ghazi, Arjumand^{1†}

¹Department of Pediatrics, University of Pittsburgh School of Medicine

²Van Andel Institute, Grand Rapids, Michigan

***Presenting author: gao63@pitt.edu, †Corresponding author: ghazia@pitt.edu.**

Diverse lines of evidence across species have suggested that reproductive health and germline integrity influence whole-body ageing and longevity. Previously, our lab demonstrated that disruption of meiosis, a germline-restricted process that is critical for reproduction and genome diversity, shortens lifespan and accelerates ageing of somatic tissues independent of the effects on fertility. Importantly, this work revealed that the young, progeric meiotic mutants exhibited transcriptional profiles strikingly similar to those observed in ageing normal animals, and shared similarities with ageing human transcriptomes. To investigate the mechanisms underlying these meiosis dysfunction phenotypes, we have performed whole-body quantitative proteomics of N2 (wild type) and three short-lived meiotic mutants, *spo-11(me44)*, *spo-11(OK79)* and *htp-3(y428)*. Using this approach, we identified a total of 7146 proteins across all four strains. Implementation of linear model for microarray data (LIMMA), allowed us to detect differentially abundant proteins and compare the elevated and depleted proteome profiles. We will present results of our characterization of candidate mechanistic regulators whose abundances are found to be altered in the meiotic mutants, including multi-omics comparative studies.

CHD7 negatively regulates histone gene expression independently of cell cycle progression in mouse embryonic stem cells

Özdemir, İsa¹; Smeal, Steven²; Henninger, Jonathan²; Hainer, Sarah²

¹Department of Biological Sciences, University of Pittsburgh

²Department of Biological Sciences, Carnegie Mellon University

CHD7 is a chromodomain-containing ATP-dependent chromatin remodeler implicated in transcriptional regulation from stem cell differentiation to neural crest development and neurogenesis, yet its role in controlling specific gene classes remains incompletely understood. Here, we investigated the impact of acute CHD7 depletion in mouse embryonic stem cells. CHD7 depletion resulted in ~30% cell death after 16 hours, whereas earlier time points showed no cell death, suggesting that it arises from progressive transcriptional dysregulation rather than immediate collapse.

To define the underlying changes, we performed RNA-seq following 8-hour and 24-hour CHD7 depletion. We observed widespread transcriptional perturbations at 8 hours that were largely resolved by 24 hours, indicating a transient transcriptional response. In contrast, S-phase-dependent canonical histone transcripts showed a persistent increase upon CHD7 depletion. Remarkably, cell cycle analysis revealed no detectable defects in cell cycle progression at either time points (early or late), indicating that histone gene dysregulation does not result from alterations in cell cycle dynamics.

Furthermore, upregulated histone transcripts exhibited extended 3' UTRs, suggesting impaired transcriptional processing and/or termination at histone loci. Canonical histone gene expression is restricted to S-phase through Histone Locus Bodies (HLBs), nuclear condensates that coordinate histone gene transcription and histone RNA processing. Together, our data suggest that CHD7 contributes to the regulation of histone gene expression by modulating HLB-associated transcriptional processing and/or termination.

Photoimmunotherapy is a safe and effective treatment for neuroblastoma in preclinical models

Pant, Arjun¹; Yip, Catherine¹; Mattioli, Milena²; Chagantipati, Bhuvitha²; Kelly, Virginia²; Choyke, Peter L.³; Hadjipanayis, Costas²; Rosenblum, Lauren¹; Kohanbash, Gary²; Malek, Marcus¹

¹Division of Pediatric Surgery, Department of Surgery, University of Pittsburgh

²Department of Neurosurgery, University of Pittsburgh

³National Institutes of Health, Bethesda, MD

Introduction: Neuroblastoma, the most common extracranial tumor in children, has high recurrence despite multimodal therapy including surgery, chemoradiotherapy, and GD2-targeted dinutuximab immunotherapy. Photoimmunotherapy (PIT) is a novel cancer treatment utilizing targeted immunotherapy agents conjugated to a near-infrared (NIR) light-activated photosensitizer to induce selective tumor death. We hypothesized dinutuximab-IR700-based PIT to be effective against neuroblastoma.

Methods: We synthesized a dinutuximab-IR700 antibody dye conjugate (ADC; 5-20 $\mu\text{g/mL}$ dose) and tested it for PIT (10-100 J/cm^2 light) on two human-derived neuroblastoma cell lines *in vitro*. Cell death was quantified by flow cytometry/microscopy. For *in vivo* testing, we used a human-derived orthotopic neuroblastoma xenograft model in Foxn1^{nu} mice. Biodistribution and safety of injected ADC was assessed with different doses over time (n=20). This informed *in vivo* PIT testing across 7 conditions (n=23): NIR-light-only, surgery-only, PIT-only (50, 75 and 100 J/cm^2 at 100 mW/cm^2), surgery+PIT (75 J/cm^2) and recurrent PIT (75 J/cm^2 twice, 5 days apart). Efficacy was measured by serial MRIs, histopathology, and immunohistochemistry.

Results: Dinutuximab-IR700 PIT induced apoptosis across cell lines. *In vivo*, highest tumor-to-background ratio (189.96) was achieved with 300 μg ADC dose at 3d, with minimal organ toxicity. PIT increased T2-signal intensity and decreased tumor size on T1-MRI in all groups 1d post-treatment, compared to light-only and control groups. Combination surgery+PIT showed greatest decrease in tumor diameter (80.7% by 1d), sustained at 2 weeks, followed by recurrent PIT (66% by 1d after second treatment). PIT monotherapy reduced tumor diameter at 1d across light doses, with regrowth at 5d (PIT-50 J/cm^2) and 2 weeks (PIT-75/150 J/cm^2). Preliminary histopathology showed loss of normal architecture with microhemorrhages.

Conclusion: Dinutuximab-IR700 PIT is a safe and effective treatment for neuroblastoma in preclinical models. Further studies are underway to confirm efficacy and reduced tumor recurrence in its use as an adjunct to surgery.

Dual photodynamic therapy and photoimmunotherapy for enhanced anti-tumor efficacy in GD2-expressing tumors

Pant, Arjun¹; Yip, Catherine¹; Chagantipati, Bhuvitha²; Mattioli, Milena²; Kelly, Virginia²; Choyke, Peter L.³; Hadjipanayis, Costas²; Rosenblum, Lauren¹; Kohanbash, Gary²; Malek, Marcus¹

¹Division of Pediatric Surgery, Department of Surgery, University of Pittsburgh

²Department of Neurosurgery, University of Pittsburgh

³National Institutes of Health, Bethesda, MD

Introduction: Photodynamic therapy (PDT) and photoimmunotherapy (PIT) are promising adjuncts to surgery for GD2-expressing tumors like neuroblastoma, osteosarcoma and glioblastoma. 5-Aminolevulinic acid (5-ALA) for PDT (established; non-selective) and Dinutuximab-IR700 for PIT (novel; GD2-targeted) are light-activated photosensitizers, which induce cell death via unique mechanisms. We hypothesized that combination PDT+PIT would synergistically enhance anti-tumor efficacy.

Methods: Dinutuximab-IR700 antibody-dye conjugate (ADC) was synthesized and tested in combination with 5-ALA on human-derived neuroblastoma (SK-N-BE(2) and NMB6) and osteosarcoma (LM7) cell lines *in vitro*. Cells were incubated with ADC (15 µg/mL) and/or 5-ALA (500 or 1000 µM), and binding/uptake confirmed by fluorescence microscopy. Cells were irradiated with 635 nm light for PDT (5-ALA; 10 or 30 J/cm²) and 689 nm light for PIT (ADC; 50 or 75 J/cm²) at 150 mW/cm². Untreated, agent-only, and light-only controls, along with single-modality PDT/PIT, were also tested. Cell death was quantified by Annexin V/propidium iodide (PI) flow cytometry and microscopy 1- and 24-hours post-therapy.

Results: Combination PDT+PIT decreased viability by >50% relative to controls in all cell lines (>70% in neuroblastoma). The addition of PIT to PDT increased cell death by up to 35% compared to PDT alone in neuroblastoma, and up to 25% in osteosarcoma. Combination therapy demonstrated a light and photosensitizer dose-sparing effect in neuroblastoma, achieving similar efficacy at lower doses than monotherapy. A 5-ALA dose-dependent cell death was observed in LM7 dual PDT/PIT. Annexin/PI staining indicated mixed apoptosis and necrosis across cell lines with combination therapy.

Conclusion: Combination PIT+PDT is superior to either alone, has light and photosensitizer dose-sparing effect, and causes greater cell damage. This treatment represents a novel approach to enable more complete eradication of GD2-expressing tumors as an effective adjunct to surgical resection.

Air Travel Challenges and Car Travel Preferences in Wheeled Mobility Device Users

Park, Sangmi¹; Matijasich, Paige²; Cooper, Rory A.¹

¹Human Engineering Research Laboratories, University of Pittsburgh

²Department of Physical Medicine & Rehabilitation, University of Pittsburgh

Despite air travel being common for both leisure and business, wheeled mobility device users (WMDs) often encounter barriers that lead to a preference for car travel. Although airlines and airports have made efforts to improve accessibility, differences by device type and barriers across travel stages remain insufficiently understood. This study examines device-specific barriers across air travel stages and evaluates their mediating role in the association between physical dependency (P-D) and car travel preference (Car) among WMDs.

This cross-sectional secondary analysis included adults (≥ 18 years) with air travel experience in the past 3 years who use a manual or power wheelchair or scooter (MW, PW, ES) as their primary mobility device. Descriptive statistics and one-way ANOVA were used to compare the number of barriers across eight air travel stages (planning, check-in, TSA screening, airport navigation, restroom use, boarding, in-flight experience, and destination). A mediation model was used with P-D as the exposure, stage-specific challenges (Challenges) as the mediator, and car travel preference as the outcome. SPSS 28 and Mplus 8.4 were used for analyses.

Data from 115 WMD users (PW=40, MW=60, ES=15) showed a significant difference in the number of barriers during check-in, with PW reporting 2.3 barriers, MW 1.47, and ES 1.67 ($F(2,112)=4.03$, $p=0.02$). The in-flight stage showed the highest overall barrier burden across all device types (PW: 47%, MW: 40%, ES: 41%). Model fit indices indicated good fit (RMSEA=0.025, TLI=0.984, CFI=0.992). Mediation analysis showed that Challenges fully mediated the relationship between P-D and Car, controlling for age and gender. P-D was significantly associated with Challenges ($\beta=0.531$, $p<0.001$), and Challenges with Car preference ($\beta=0.406$, $p<0.01$).

Understanding differences in air travel challenges by device type, as well as across travel stages, may help improve support for more accessible air travel among WMDs.

Party Norms Prevail: Instrumental Voting and Resistance to Crossover Voting

Park, Yul Min¹; Woon, Jonathan²

¹Department of Political Science, University of Pittsburgh

²Department of Political Science, Department of Economics (secondary), University of Pittsburgh

Opportunities for crossover voting—when partisan voters participate in the opposing party’s primary elections to influence its nominee—pose a puzzle for theories of strategic behavior. We present evidence from two preregistered studies showing that instrumental logic explains much, but not all, of voter behavior. Consistent with standard theories of instrumental voting, support for crossover voting rises when voters view one candidate much more negatively than another and when they believe a candidate is more likely to win the general election. Yet experimental primes designed to heighten concern about authoritarian threats had no measurable effect, suggesting limits to informational interventions when preferences are already firmly established.

More importantly, we introduce a novel account of party voting norms: internalized beliefs that crossing party lines is inappropriate. These norms significantly suppress willingness to cross over, even among voters with strong instrumental incentives to do so, and they appear clearly in respondents’ open-ended explanations, which frequently describe crossover voting as wrong, unfair, or inconsistent with how party elections should work.

Together, the findings show that strategic behavior is shaped by not only instrumental incentives but also normative commitments about how democratic elections should work. More broadly, the study highlights how internalized electoral norms can constrain strategic adaptation even in usually high-stakes and strategically transparent electoral contexts.

Preclinical Investigation of Ketogenic Diet and Vadadustat as Therapeutic Approaches in *TPI^{E105D/null}* and *TPI^{E105D/E105D}* Mouse Models of Triosephosphate Isomerase Deficiency

Patel, Priyal^{1,2}; Satterfield, Sidney^{1,2}; Roberts, Presley^{1,2}; Coleman, Grace^{1,2}; Vogt, Andreas³; Homanics, Gregg⁴; Palladino, Michael^{1,2}

¹Department of Pharmacology & Chemical Biology, University of Pittsburgh

²Pittsburgh Institute for Neurodegenerative Disease, University of Pittsburgh

³Department of Computational & Systems Biology, University of Pittsburgh

⁴Department of Anesthesiology & Perioperative Medicine, University of Pittsburgh

Background: Triosephosphate Isomerase Deficiency (TPI Df) is a rare autosomal recessive glycolytic enzymopathy manifesting during infancy with hemolytic anemia, progressive neurodegeneration, and early death. The most common mutation, *TPI^{E105D}*, drives protein instability and loss of glycolytic function. We hypothesized that stabilizing the mutant TPI protein could preserve residual enzymatic activity, and dietary strategies could mitigate the disease by glycolytic compensation, reducing methylglyoxal (MGO), or promoting ketosis.

Methods: Using TPI Df mouse models, *TPI^{E105D/null}* and *TPI^{E105D/E105D}*, we explored two therapeutic strategies. *TPI^{E105D/null}* mice (~95% reduced TPI protein; shorter expectancy) were randomized at postnatal day 22 into standard chow, control, and ketogenic diet, with daily survival monitoring, and rotarod performance every 5-days. For the pharmacological approach, patient-derived fibroblast cells (FB303) homozygous for *TPI^{E105D}* mutation were treated with vadadustat (100 μ M, 24 h), and TPI protein levels were measured by western blot. *TPI^{E105D/E105D}* mice (milder phenotype; longer lifespan) received oral vadadustat (175 mg/kg) and/or vehicle (0.25% HPMC and 0.1% Tween 80) for 20 days, and efficacy was assessed by rotarod performance and TPI protein levels measured via western blot. **Result and Conclusion:** Ketogenic diet significantly extended the lifespan of *TPI^{E105D/null}* mice relative to control or chow groups ($p = 0.0012$), with ~50% surviving beyond day 270, suggesting benefit via glycolytic bypass, promoting ketosis, and/or reducing MGO production. Keto-fed mice showed a non-significant ($p = 0.0964$) improvement in motor performance. In FB303 fibroblasts, vadadustat showed a ~45% increase in TPI protein levels ($p = 0.0002$), demonstrating its ability to stabilize mutant *TPI^{E105D}*. While in *TPI^{E105D/E105D}* mice, vadadustat significantly ($p=0.043$) improved motor function at day 40 and elevated TPI protein levels in the liver ($p=0.0002$), kidney (0.0002), muscle ($p=0.0104$), and with no effect in brain ($p=0.0878$), suggesting peripheral stabilization of mutant TPI and may modestly improve muscle function, supporting its therapeutic potential in TPI Df.

SMARCD3 Regulates Cerebellar Development and Drives Changes in Medulloblastoma

Zou, Han¹; Patel, Yash¹; Hu, Baoli¹

¹Department of Neurological Surgery, University of Pittsburgh

Introduction / Purpose

Medulloblastoma (MB) is the most common malignant pediatric brain tumor and arises in the cerebellum. Among its four molecular subgroups (WNT, SHH, Group 3, and Group 4), Group 3 MB is the most aggressive and is frequently associated with metastasis and poor prognosis. The developmental origins and molecular mechanisms underlying Group 3 MB dissemination remain incompletely understood. We investigated the role of SMARCD3 (BAF60C), a core component of the SWI/SNF chromatin-remodeling complex, in cerebellar development and Group 3 MB progression.

Methods

SMARCD3 expression was analyzed in patient datasets and developing cerebellar tissue. Survival and pathway analyses were performed to determine clinical and molecular associations. Conditional Smarcd3 knockout mice targeting Purkinje cells (PCs) were generated to evaluate in vivo function. Behavioral assays, histological staining, and dendritic morphology analyses were conducted to assess cerebellar structure and motor coordination.

Results

SMARCD3 was highly expressed in Group 3 MB and in Purkinje cells during cerebellar development. Elevated SMARCD3 expression correlated with poor patient survival, metastatic phenotype, and activation of the Reelin signaling pathway, a key regulator of PC migration and positioning. Conditional deletion of Smarcd3 in PCs resulted in early lethality; surviving mice exhibited impaired motor coordination and balance. Histological analyses revealed disrupted PC alignment and reduced dendritic arborization, confirming a critical role for SMARCD3 in PC migration, positioning, and maturation. Mechanistically, SMARCD3-associated SWI/SNF complexes regulated Reelin pathway activity during cerebellar development.

Conclusion

Our findings demonstrate that a SMARCD3-dependent neurodevelopmental chromatin-remodeling program governing Reelin-mediated PC organization is co-opted to promote metastatic dissemination in Group 3 MB. This study establishes a mechanistic link between cerebellar development and tumor metastasis and identifies SMARCD3 as a potential therapeutic target in high-risk MB.

Lifetime adversity's indirect effect on alcohol use via sleep disturbances among juvenile-justice involved youth

Pham, Holly T.¹; Quick, Allysa¹; Wallace, Meredith L.^{1,2}; Mulvey, Edward¹; Miller, Elizabeth³; Levenson, Jessica C.^{1,3}

¹Department of Psychiatry, University of Pittsburgh

²Department of Biostatistics, University of Pittsburgh

³Department of Pediatrics, University of Pittsburgh

Adolescent alcohol use is associated with increased risk of preventable death among youth and for physical and behavioral health problems across the lifespan. Juvenile-justice involved youth (JJY) have much higher rates of alcohol use than their non-juvenile justice-involved peers and are more likely to have been exposed to adversity, a key risk factor for adolescent alcohol use. Poor sleep health may underlie the link between adversity and alcohol use, making it a potential intervention target for JJY at risk for adolescent alcohol use. This study investigated if lifetime adversity was related to alcohol use via poor sleep health among JJY.

Self-reported data were provided by 100 participants (ages 13-18 years) from a cross-sectional study on sleep among adolescents involved in the United States juvenile justice system. Models assessing the indirect effect of lifetime adversity on past-6-month alcohol use via various sleep health indicators (i.e., sleep duration, total sleep time, time in bed, sleep efficiency, social jetlag, sleep-related impairment, and sleep disturbances) were tested with the PROCESS macro using bias-corrected confidence intervals with 5000 bootstraps. All models controlled for age, sex, and community versus residential placement.

Greater number of lifetime adverse experiences were indirectly related to higher likelihood of alcohol use in the past 6 months via higher levels of sleep disturbances ($b = 0.10$, 95% CI [0.01, 0.34]). There were no significant indirect effects via other sleep health indicators.

Exposure to adversity may lead to sleep disturbances, such as insomnia and poor sleep quality, which then may in turn increase alcohol use risk among JJY. Future longitudinal work investigating these associations is needed. This study's findings support calls to consider and potentially target sleep health in the context of increasing resilience among youth at risk for problematic alcohol use, particularly JJY with a history of adversity.

Objective and subjective socioeconomic status and cognitive function in lower income adults

Phillips, Meredith L.¹; Hill-Jarrett, Tanisha,²; Troxel, Wendy M.³; Dubowitz, Tamara¹; Rosso, Andrea L.¹

¹Department of Epidemiology, School of Public Health, University of Pittsburgh

²Memory and Aging Center, Department of Neurology, University of California San Francisco

³Division of Social and Economic Well-Being, RAND Corporation

The risk of cognitive impairment is elevated for individuals with lower socioeconomic status (SES). Although SES operates through multiple pathways to affect cognition, it is frequently measured using a single variable.

Using participants from Think PHRESH, a continuation of the Pittsburgh Hill/Homewood Research on Neighborhood Change and Health (PHRESH) study, we estimated associations between two SES measures in 2018 and global cognitive function in 2023. Our exposures were household income and subjective SES (social standing ladder). Our outcome was the Modified Mini-Mental State Examination (3MS). We fit linear regression models estimating associations between SES and 3MS, controlling for neighborhood, age, sex, education, and household income.

Our sample (N=475) is 82% female, 94% Black, 12% with less than high school diploma, with a mean age of 64. We found household income was positively associated with 3MS (β : 0.09, 95% CI: 0.05, 0.13), whereas higher subjective SES was associated with lower 3MS score (β : -0.85, 95% CI: -1.30, -0.41). Following this unexpected result, we added an SES*education interaction term. We found no significant interaction with household income. For subjective SES, the negative association was primarily driven by participants with less than a high school diploma. In this group, higher subjective SES is associated with lower 3MS (β : -2.84, 95% CI: -4.51, -1.17). We found no association among those with higher levels of education.

In this low-income, predominantly Black sample, objective indicators of SES may be more predictive of general cognitive functioning, whereas the relationship between subjective indicators and cognition was more complex.

HPV (+) Tumor derived Extracellular Vesicles (sEV) Transfer HPV Genes to Recipient Cells Triggering Cellular Reprogramming.

Pinero, Carlos¹; Beatty, Pamela¹; Whiteside, Theresa¹

¹Department of Pathology, School of Medicine, UPMC Hillman Cancer Center

This research aims to study the presence of HPV genes and proteins in small extracellular vesicles (sEV) produced by HPV (+) head and neck squamous cell carcinoma (HNSCC). I plan to investigate the extent to which sEV carrying HPV DNA can transfer it horizontally to other cells and induce cellular reprogramming thereby promoting cancer genesis and progression. It intends the investigation of the role tumor-derived sEV (TEX) play as biomarkers of diagnosis and prognosis of HNSCC. The first aim (A) isolated TEX from plasma of patients with HPV (+) HNSCC will be tested for the presence of HPV16 proteins by WBs and lysed for proteomic analyses and evaluated for the presence of viral peptides (E6, E7, E5, E2). In (B) the isolated sEV will be tested for HPV16 proteins by WBs and used for DNA extraction and analyses for the presence of viral DNA by digital PCR (dPCR). We expect to show that tumor-associated HPV DNA can be detected and sequenced in clinical samples of HPV (+) sEV. The second specific aim is to examine the mechanisms used by HPV (+) TEX to transfer the HPV proteins and DNA to diver types of recipient cells *in vitro*. Coincubations of the HPV (+) sEV produced by HPV (+) OSCC cell lines with HPV (-) tumor cells, non-tumor cells and immune cells will determine the extent to which sEV transfer viral DNA to recipient cells and reprogram their functions. We will look at the expression of the viral oncogenic drivers (E5, E6, E7) as well as the transcriptional regulatory protein E2 in recipient cells. We will determine the ability of recipient cells reprogrammed by TEX as in (A) to produce sEV carrying viral DNA and to disseminate the virus thereby promoting HPV-mediated carcinogenesis.

Rap1b Activates AC9 to Drive the Intracellular cAMP Wave

Pinto, Alipio¹; Pizzoni, Alejandro¹; Zhang, Xuefeng¹; Altschuler, Daniel L.¹

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh

Gs-coupled GPCR signaling generates cAMP in distinct spatial waves that begin at the plasma membrane (PM) and propagate into intracellular compartments. Although the molecular basis of the PM wave is well established, the mechanisms driving the intracellular cAMP wave remain unclear. Here, we identify the small GTPase Rap1b as a direct activator of the endosomal adenylyl cyclase 9 (AC9), uncovering a mechanism for intracellular cAMP production. Like Gs, Rap1b-GTP binds the C2 domain of AC9 and allosterically stimulates its catalytic activity in vitro and in cells. By using AC9 mutants that selectively impair Rap1b or Gs binding, we show that the Rap1b-AC9 signaling unit is required for the intracellular cAMP wave, revealing a new layer of spatial regulation in cAMP signaling.

Head-to-head comparison of tau PET tracers across the Alzheimer's disease spectrum

Povala, Guilherme¹; Bellaver, Bruna¹; Lussier, Firoza Z.¹; Amaral, Livia¹; Bauer-Negrini, Guilherme¹; Ferreira, Pamela C.L.¹; Rocha, Andreia¹; Ruppert, Emma¹; Medeiros, Marina S.¹; Jagust, William J.²; Masdeu, Joseph³; Fortea, Juan⁴; Tudorascu, Dana L.¹; Soleimani-Meigooni, David N.^{2,5}; Lowe, Val⁶; Oh, Hwamee⁷; Pascual, Belen³; Gordon, Brian A.⁸; Rosa-Neto, Pedro⁹; Baker, Suzanne²; Pascoal, Tharick A.^{1,10}

¹Department of Psychiatry, University of Pittsburgh

²Lawrence Berkeley National Laboratory

³Department of Neurology, Houston Methodist Research Institute

⁴Sant Pau Memory Unit, IR SANT PAU, Hospital de la Santa Creu i Sant Pau

⁵Memory and Aging Center, University of California San Francisco

⁶Department of Radiology, Mayo Clinic

⁷Department of Psychiatry and Human Behavior, Brown University

⁸Department of Radiology, Washington University in St. Louis

⁹Translational Neuroimaging Laboratory, Douglas Research Institute, McGill University Research Centre for Studies in Aging

¹⁰Department of Neurology, University of Pittsburgh

Background: Tau PET imaging is an important tool for AD clinical trials, diagnosis, and biomarker research. Here, we present the primary cross-sectional outcomes from the HEAD study comparing Tauvid and MK6240 uptake.

Methods: We enrolled 775 individuals, 682 had complete Tauvid, MK6240 and A β PET, including 38 young adults (aged 18-28), 362 cognitively unimpaired elderly (CU) and 282 cognitively impaired (CI). Tau positivity in the medial temporal lobe (MTL) and neocortex was defined as uptake exceeding the mean + 2.5 SD of young adults. Prevalence ratios (PR) were estimated with Poisson regression with robust variance adjusting for Centiloid. The number of participants needed to identify one tau-positive was defined as the inverse of the observed positivity rate (1/prevalence).

Results: In CU, MTL positivity was detected in 6.35% with Tauvid and 14.92% with MK6240, corresponding to a twofold higher prevalence for MK6240 (All CU: PR=2.35, $p<0.0001$; CU A β +: PR=2.43, $p=0.001$). Among CI, MTL positivity was 52.8% versus 65.3% (All CI: PR=1.23; $p=0.0002$; CI A β +: PR=1.19, $p=0.0002$), and neocortical positivity 16.3% versus 28.4% (All CI: PR=1.74; $p=0.0002$; CI A β +: PR=1.74, $p<0.0001$). To identify one MTL-positive case, 6.3 CU A β + individuals would need to be scanned with Tauvid and 2.6 with MK6240. For neocortical positivity, 10.8 and 4.2 MCI A β +, and 2.5 and 1.6 AD dementia participants would need to be scanned with Tauvid and MK6240, respectively. The tracers had 87% of MTL-positivity agreement, 11.6% were positive only with MK6240 and 1.4% only with Tauvid. MK6240-only individuals showed higher A β burden, APOE ϵ 4 carriership, and worse memory scores than dual-negative participants.

Conclusions: Our results highlight that quantitative detection of tau pathology differs between tracers, with MK6240 detecting substantially more MTL and neocortical tau pathology than Tauvid. MK6240-only participants had higher Centiloid and worse cognitive scores, supporting the pathological relevance of MK6240-only uptake.

Global Perspectives on Trauma Training Availability and Trainee Competency

Prakash, Krithika¹; Jenkins, Dan²; Picot, Laura³; Alves, Virgínia⁴

¹Department of Psychiatry, University of Pittsburgh

²Department of Psychology, University of Fribourg

³Bordeaux Neurocampus, Université de Bordeaux

⁴School of Psychology, Universidade do Minho

Background and Aims: Psychological traumatic stress is a significant global health issue, yet specialized training remains highly variable and often lacks validated assessments to measure clinical competency. While training needs are widespread, there is a distinct gap in standardized tools to evaluate the readiness of trainees in various international contexts. This international, trainee-led pilot study aims to evaluate trainee experiences, the availability of trauma-specific training, and needs across different regions..

Method: This international study involves project leads from France, Portugal, Switzerland, and the USA. We aim to recruit a total of 400 trainees (100 per country), including graduate students, residents, and fellows across disciplines (e.g., psychology, social work, medicine, and nursing).

To understand their trauma training, the survey will ask about trauma-specific clinical hours and the availability of trauma training in their region (e.g., formal certification, supervision, or webinars). The study will include a 20-item trauma clinical competency self-assessment, that was developed by the research team based on APA guidelines on Trauma Competencies for Education and Training (APA, 2025). We have used behaviorally anchored items derived from these recommendations to evaluate specific competencies in 1) trauma knowledge, 2) clinical skills, and 3) reflexive practice. We are currently in the data collection phase of the study.

Regression analyses will be employed to examine the relationship between training availability, perceived needs, and competency of trainees across the various regions and disciplines. Factor analysis will be used to validate the psychometric properties of the new trauma competency scale.

Conclusions: This study represents a critical step toward understanding the landscape of trauma training internationally. By examining the relationship between clinical exposure, needs, and self-reported competence, this research will provide empirical support for professional guidelines and may inform evidence-based trauma curriculum.

A knock-in strategy identifies Prx1 expressing cells as progenitors of osteocytes, PDL cells, and cementoblasts

Rasheed Issa, Dalia^{1,2}; Geng, Xue Hui²; Intini, Giuseppe^{1,2,3}

¹Department of Periodontics and Preventive Dentistry, University of Pittsburgh

²Pittsburgh Center for Craniofacial Regeneration, University of Pittsburgh

³Hillman Cancer Center, University of Pittsburgh

Objectives: Postnatal paired-related homeobox 1 expressing cells (pnPrx1⁺ cells) contribute to dental tissue development; however, their role in periodontal homeostasis and regeneration remains unclear. This study aimed to determine whether a Cre recombinase knock-in at the Prx1 locus provides a more accurate delineation of these cellular homeostatic and regenerative processes.

Methods: For homeostasis, transgenic Prx1-CreER-GFP/tdTomato and Prx1-CreER knock-in/tdTomato mice were allocated into three groups (n=3): 0, 1, or 2 doses of tamoxifen administration. Mandibles were harvested at day 0 and at 2- and 3-days post-injection. For regeneration, a fenestration periodontal defect ($\approx 1 \times 2 \times 0.5$ mm) was created. Mice were assigned to two groups and euthanized at 7- or 30-days post-surgery. Each group included tamoxifen treated (3 doses, starting 2 days pre-surgery and every 48 hours thereafter) and controls (n=3/subgroup). For long-term healing, Prx1-CreER-knock-in/tdTomato mice were administered tamoxifen and euthanized at 60-days and 90-days post-surgery (n=3/group). Immunohistochemistry was performed using anti-Bone Sialoprotein (BSP) at all post-surgery time points, and anti-Osterix (SP7) at 60- and 90-days post-surgery.

Results: During homeostasis, tdTomato⁺ progeny of pnPrx1⁺ cells were observed in the knock-in mice, whereas the transgenic mice exhibited minimal detectable tdTomato⁺ cells. Following injury, tdTomato⁺ cells were significantly more abundant in the knock-in mice, distributed throughout the newly forming periodontal ligament (PDL) and alveolar bone (AB), and concentrated near the defect area. At 60- and 90-days post-surgery, tdTomato⁺ cells in AB were identified as osteocytes. Strong tdTomato⁺ signal was observed throughout the regenerated PDL, closely associated with the newly formed cementum. Increased cementum thickness and mineralization corresponded with elevated BSP expression. Co-localization of tdTomato⁺ and SP7⁺ cells at defect sites indicated differentiation into cementoblasts.

Conclusion: The knock-in model enables improved assessment of pnPrx1⁺ cell contributions to the periodontal maintenance and regeneration, demonstrating their differentiation into cells of the AB, PDL, and cementum.

Physiological Load, Neuromuscular Performance, Body Composition and Inflammatory Responses During 4 Weeks of US Marine Corps Mountain Warfare Training

Rawls, Bobby G.¹; Kargl, Christopher K.¹; Redinger, Allen L.¹; Rosenblum, Lily J.¹; Lewellen, Jennifer N.¹; Lovalekar, Mita¹; Mroz, Kelly H.¹; Koltun, Kristen J.¹; Martin, Brian J.¹; Nindl, Bradley C.¹

¹Neuromuscular Research Laboratory/Warrior Human Performance Research Center, Department of Sports Medicine and Nutrition, University of Pittsburgh

Background:

Extended training without workload monitoring puts military personnel at risk for overtraining and maladaptation characterized by decrements in performance, hormonal dysregulation, and increased injury risk. The purpose of this study was to investigate neuromuscular, endocrine, and body composition changes in response to an intensive 4-week Mountain Exercise (MTX) designed to prepare Marines for operations in treacherous environments, leveraging wearable devices to characterize course-long workload.

Methods:

47 male Marines (Age:21.6±3.5yrs, BMI:27.1±3.4kg/m²) were assessed via countermovement jump (CMJ), serum hormone concentrations (insulin-like growth factor I [IGF-I], sex hormone binding globulin [SHBG], total testosterone [TT], interleukin-8 [IL-8], IL-10) and body composition Pre/Post MTX. Garmin Instinct watches were worn to quantify workload for the course duration. Paired T-tests assessed timepoint differences and Cohen's d was used to calculate effect size.

Results:

Workload: Mean distance traveled was 9.3±3.8km/d; mean elevation gain of 83.2± 3.6m/d; mean caloric expenditure was 3155.8±533.3kcal/d with 3.1±1.0hrs/d of moderate to vigorous activity. *Body Composition:* Body mass, lean mass, and fat mass decreased by 6.2% ($p<.001$, ES=-2.42, 95% CI[-5.93– -4.52kg]), 4% ($p<.001$, ES=-0.91, CI[-3.74– -1.78kg]) and 15% ($p<.001$, ES=-0.84, CI[-2.92– -1.30kg]), respectively. *CMJ:* Concentric mean force, eccentric mean force, and eccentric + concentric power/time decreased by 7.6% ($p<.001$, ES=-1.06, CI[-157.6– -89.46N]), 5.9% ($p<.001$, ES=-2.48, CI[-54.17– -42.68N]), and 10% ($p=.001$, ES=-0.50, CI[-230.33– -60.77W/s]), respectively. *Biochem:* IGF-I and TT levels decreased by 31.8% ($p<.001$, ES=-0.96, CI[-39.69– -22.76ng/mL]) and 8.8% ($p=0.02$, ES=-0.5, CI[-15.37– -1.81ng/dL]), respectively, while SHBG levels increased by 41.5% ($p<.001$, ES=1.00, CI[27.06–57.62 nmol/L]) and IL-8 and IL-10 increased by 12% ($p=0.049$, ES=0.32, CI[0.01–25.57 pg/mL]) and 18.3% ($p<0.01$, ES=0.5, CI[6.11–31.80 pg/mL]), respectively.

Conclusion:

There was a maladaptive response exhibited by decreases in total body mass, lean mass, fat mass, and CMJ force/power capabilities in conjunction with elevated SHBG and markers of inflammation with a concurrent decline in IGF-I and TT.

Cortical Spreading Depression Evoked Activation of Dural Nonmyelinating Schwann Cells

Reddy, Aravind T.¹; Gibson, Gregory A.²; Watkins, Simon C.²; Gold, Michael S.¹

¹Department of Neurobiology, University of Pittsburgh

²Department of Cell Biology, University of Pittsburgh

Migraine affects roughly 15% of the global population, is a leading cause of years lived with disability, and is ~3x more common in women. Although classically viewed as a brain disorder, migraine pain arises from activation and sensitization of trigeminovascular afferents within a complex meningeal microenvironment. The purinergic hypothesis of migraine proposes that ATP released during attacks acts on P1 and P2 receptors to drive meningeal nociception. Subsequent work has highlighted ATP–P2X3 signaling in trigeminal afferents and the broad expression of purinergic receptors in neurons, vascular cells, and immune cells in the meninges. Nonmyelinating Schwann cells (NMSCs) ensheathing meningeal afferents are well positioned to sense extracellular ATP and modulate nociceptor excitability. Building on these observations, we asked whether NMSCs might serve as an intermediary target linking cortical spreading depression (CSD), the phenomenon underlying aura preceding the headache in a subpopulation of migraineurs, to nociceptor sensitization. In Trpv1-Cre-GCaMP8s mice, we combined KCl-evoked CSD with in vivo and ex vivo calcium imaging of dural meninges. CSD reliably evoked large, rapid-onset Ca²⁺ transients in dural NMSCs aligned with fibers. Over time, the fraction of activated NMSCs continued to increase. These data provide the first direct evidence that CSD robustly activates dural NMSCs. Ongoing studies are testing whether P2 receptor blockade and inhibition of intracellular Ca²⁺ stores attenuate NMSC activation and associated meningeal nociceptor activity. Funded by the Bloch Foundation and the National Institutes of Health (T32NS073548 and R01NS122784).

Achilles Tendon and Myotendinous Macro- and Micro-Morphology, Quality, and Performance Adaptations to 10 Weeks of U.S. Marine Corps Officer Candidate School Training

Redinger, Allen¹

¹Neuromuscular Research Laboratory/Warrior Human Performance Research Center, Department of Sports Medicine and Nutrition, University of Pittsburgh

Background: U.S. Marine Corps (USMC) Officers demonstrate elevated Achilles tendon (AT) injury rates compared to personnel across other branches. To commission, Candidates must complete 10 weeks of Officer Candidates School (OCS), which may influence myotendinous injury risk before service. However, myotendinous morphology and performance adaptations to such training are unknown. **Purpose:** Evaluation of myotendinous morphology, collagen fiber organization, and performance using ultrasound, spatial frequency analysis (SFA), and jump/landing forces in USMC Officer Candidates undergoing 10-weeks of OCS. **Methods:** Candidates (n=104 males, age 26.0±3.2 years, BMI 25.9±4.3 kg/m²) were assessed pre-/post-training. B-Mode ultrasonography was used to assess free tendon volume (tV) and echogenicity (tEI, indicative of collagen fiber concentration), and physiological cross-sectional area (pCSA, functional muscle architecture) of the medial gastrocnemius. SFA assessed AT collagen fiber spacing (PSFR), organization (P6), and density (Amax). Additionally, force plate countermovement jumps evaluated jump/landing peak forces, stiffness, and rate of force development (RFD). Wilcoxon signed-rank test compared pre- to post-OCS differences due to non-normally distributed data, Cohen's *d* effect sizes were calculated, and $\alpha=0.050$. **Results:** Candidates demonstrated an increase in tV (+4.5%; $p<0.001$; $d=0.28$), a decrease in tEI (-1.4%; $p=0.005$; $d=0.25$), PSFR (-1.7%; $p=0.004$; $d=0.32$), and Amax (-2.7%; $p=0.006$; $d=0.35$) and an increase in P6 (+0.3%; $p=0.046$; $d=0.23$); indicating increased intra-tendinous edema and collagen fiber spacing and disorganization. This coincided with reductions in jump peak force (-7.0%; $p<0.001$; $d=0.41$), stiffness (-9.7%; $p<0.001$; $d=0.26$), and RFD (-26.7%; $p<0.001$; $d=0.26$), despite increased medial gastrocnemius pCSA (+6.2%; $p=0.001$; $d=0.36$). **Conclusions:** These findings indicate that degenerative adaptations of the AT (mimicking similar traits found in tendinopathy) were observed in response to 10 weeks of OCS. Additionally, subsequent decrements in force production and development were observed, despite an increase in gastrocnemius pCSA. Together, these findings may suggest an increased risk of myotendinous injury in new Officers post OCS.

Compounding Disasters and Community Economics: Children Living in Poverty Following the 2021 Kentucky Tornado Outbreak

Rienne, Gavin¹

¹Center of Excellence in Maternal and Child Health, University of Pittsburgh

Background: Rural communities face disproportionate disaster-related health burdens but remain underrepresented in disaster recovery research. The December 2021 Kentucky tornado outbreak devastated multiple counties across western and central Kentucky, including Graves County, where the city of Mayfield was devastated. These communities were struck during an ongoing COVID-19 recovery period, creating a compounding disaster context that may have exacerbated economic hardship and worsened longstanding vulnerabilities. Despite the breadth and impact of the disaster, the population-level economic consequences for children remain unexamined.

Objective: This study examines trends of children living in poverty in tornado-affected Kentucky counties from 2018 through 2024, capturing pre-disaster baseline, the COVID-19 period, acute disaster impact, and post-disaster recovery. We assess whether the compounding burden of COVID-19 and acute tornado exposure produced detectable and persistent economic ramifications in affected rural communities, and whether social and economic community indicators buffered population-level impact.

Methods: We employ a repeated cross-sectional pooled design with county and year fixed effects using Annie Casey Foundation KIDS Count Data Center data for 11 tornado-affected Kentucky counties and matched 1:2 to comparable unaffected Kentucky counties on 2019 population and median household income. A year-by-year event-study specification examines annual deviations in children in poverty to the 2021 event year over a 7-year panel ($k-3$ through $k+3$). Covariates are selected via LASSO regression from the KIDS Count and Robert Wood Johnson Foundation County Health Rankings data, matched by county. Standard errors are clustered at the county level.

Preliminary Findings: Analysis is ongoing. Preliminary findings will be presented.

Conclusion: This study addresses a significant research gap by examining the effects of compounding disaster events on children's well-being. Findings will increase our understanding of rural community disaster response and resilience under compounding events and carry direct implications for disaster preparedness, resource allocation, and policy for rural populations.

Mechanism of action of methotrexate (MTX) in boosting retinal function in autosomal dominant retinitis pigmentosa

Rizwan, Saira^{1,2}; Sheng, Li^{1,2}; Chen, Yuanyuan^{1,2}

¹UPMC Mercy Vision Institute, Department of Ophthalmology, University of Pittsburgh

²Department of Pharmacology and Chemical Biology, University of Pittsburgh

Purpose: Rhodopsin can misfold, mislocalize, or aggregate due to mutations. These traits can overwhelm the proteostasis capability of rods, leading to rod cell death and eventually retinal degeneration including autosomal dominant retinitis pigmentosa (adRP). There is no effective treatment for adRP. We previously found that MTX effectively clears out misfolded rhodopsin mutants in vitro without affecting WT rhodopsin. MTX significantly enhances rod function. Our pre-clinical study and recently published clinical trial in RHO-adRP patients show that MTX significantly increases rod function either by systemic or intravitreal administration. This study aims to understand the mechanism of action of MTX, an anti-folate drug candidate, in improving rhodopsin homeostasis and retinal function in adRP.

Methods: We performed RNA sequencing and immunoblots to evaluate AMPK phosphorylation, mitochondrial biogenesis, and localize adenosine receptor expression in mouse model treated with MTX for 24 hours. We administered MTX to one-month-old *RHO*^{P23H/+} and control mice and performed ERG to evaluate visual function. We then processed retinal tissue for metabolic pathway analysis, gene ontology, and combined molecular pharmacology assays.

Results: Both scotopic and photopic waves increased 24h after a single systemic dose of MTX in *RHO*^{P23H/+}. Immunoblots from MTX treated *RHO*^{P23H/+} retinæ show increased total rhodopsin level, compared to vehicle control group. RNA sequencing revealed that a low dose of MTX significantly downregulated the expression of genes involved in PPAR pathway, while AMPK pathway was upregulated in the *RHO*^{P23H/+} but not WT mouse retinæ. These results suggest that the efficacy of MTX is more specific to disease model, which could explain increased ERG responses.

Conclusion: Systemic MTX administration at a low dose enhances autophagy through AMPK and adenosine signaling pathways. Low dose MTX treatment may be a therapeutic strategy for boosting retinal functions in RHO-adRP.

Premature senescence: a potential driver of alveolar bone destruction in periodontal disease.

Robert, Nadine¹; Lacin, Nursima¹; Ki, Yejin¹; Kobyra, Julie²; Sfeir, Charles^{1,2}

¹Center for Craniofacial Regeneration, School of Dental Medicine, University of Pittsburgh

²Swanson School of Engineering, University of Pittsburgh

Periodontal disease (PD) is a chronic inflammatory disease resulting from a complex interplay between pathogenic bacteria in dental plaque and the host immune response. Recent studies have shown that bacterial aggression in PD can induce DNA damage, leading to cellular senescence. Senescence, long considered a hallmark of aging, is characterized by a permanent state of cell arrest in which cells remain metabolically active and secrete the senescence-associated secretory phenotype (SASP) factors. However, the role of senescence in PD initiation and progression in young individuals remains poorly defined. We hypothesize that acute bacterial aggression in PD triggers premature senescence through various mechanisms, leading to a persistent inflammatory state that drives alveolar bone loss. To address our hypothesis, we tied a 5.0 silk ligature around the second maxillary molars of 6- 8-week-old Balb/c mice for 4 or 10 days. We explored the mechanisms driving cellular senescence in gingival tissues using single-cell RNA sequencing analysis. Healthy mice were used as controls. PD induction was confirmed by micro-CT analysis. We examined the canonical pathways related to senescence in PD at D4 and D10, compared with those in healthy mice, using Ingenuity Pathway Analysis tool. Senescence, unfolded protein response, DNA damage response, mitochondrial dysfunction, and biogenesis were among the significant pathways activated or inhibited in PD compared with healthy state. Differential Expression Visualization using ShinyCell App analysis tool demonstrated upregulation of genes related to mitochondrial oxidative stress, cell cycle arrest, lipofuscin, endoplasmic reticulum stress, paracrine senescence, and senescence-associated β -galactosidase in PD compared to healthy group. Downregulation of *Trp53* gene in PD groups suggests a persistence of damaged cells, chronic inflammation, and SASP secretion, which was confirmed. Our preliminary findings suggest that premature senescence may drive alveolar bone destruction during PD initiation and progression by creating a sustained permissive chronic inflammatory environment.

Tracking Tau Spread: MK6240 Assigns Higher Braak Stages Than Flortaucipir

Rocha, Andreia¹; Soares, Carolina¹; Ruppert, Emma¹; Madeiros, Marina S.¹; Bellaver, Bruna¹; Ferreira, Pamela C. L.¹; Povala, Guilherme¹; Amaral, Livia¹; Bauer-Negrini, Guilherme¹; Lussier, Firoza Z.¹; Rodrigues, Matheus S.¹; Masdeu, Joseph C.²; Tudorascu, Dana L.¹; Soleimani-Meigooni, David N.³; Lowe, Val J.⁴; Oh, Hwamee⁵; Pascual, Belen²; Gordon, Brian A.⁷; Rosa-Neto, Pedro²; Baker, Suzanne L.⁸; Pascoal, Tharick A.¹

¹Department of Psychiatry, School of Medicine, University of Pittsburgh

²Houston Methodist Research Institute

³University of California, San Francisco

⁴Department of Radiology, Mayo Clinic

⁵Brown University

⁶Washington University in St. Louis

⁷McGill University

⁸Lawrence Berkeley National Laboratory

Background: In vivo Braak staging using tau-PET imaging is a widely used framework for classifying Alzheimer's disease (AD) tau pathology progression. However, consistency of Braak staging across different PET radiotracers have not yet been confirmed, particularly given tracer-specific differences in binding sensitivity and specificity. This study directly compares Braak staging between the two most extensively used tau PET tracers, Flortaucipir and MK6240, in the same individuals in a head-to-head design. **Methods:** We assessed 644 participants across the AD continuum who underwent MK6240- and Flortaucipir-PET, MRI, A β -PET, and clinical evaluations. Participants were assigned to the seven (0-VI) Braak framework based on median SUVRs from Braak-like ROIs. Tracer-specific positivity cut-offs were defined as the mean + 2 SD of the middle-aged CU A β - reference group (≤ 65 years, Centiloid < 10). Braak stage concordance was evaluated between tracers; discordant individuals were visually inspected to assess off-target binding. **Results:** Group-level Braak stage distributions appeared broadly similar between tracers, though MK6240 identified a slightly higher proportion of positive cases and more individuals at advanced Braak stages. However, when compared at the individual level, substantial discrepancies emerged: only about 60% of A β ⁺ participants were assigned the same Braak stage. Among discordant cases, 89% showed higher Braak classification on MK6240, whereas only 11% were higher on Flortaucipir (Fig2). Visual inspection revealed that most Flortaucipir elevations were related to off-target uptake, whereas MK6240 elevations corresponded to binding consistent with tau pathology (Fig3). **Conclusion:** Although overall Braak stage distributions appeared roughly comparable between tracers, individual-level analyses revealed considerable discordance (approximately 40% of the cases), with MK6240 frequently assigning higher Braak stages to the same individuals. These findings suggest that MK6240 may capture tau deposition earlier along the Braak continuum, leading to a systematic tendency to classify a greater proportion of individuals at higher Braak stages.

Global loss of *Crebrf* in mice results in hyperglycemia despite resistance to diet-induced obesity in part due to reduced functional beta cell mass

Mary G. Rossillo¹, Polly E. Mattila², Zana M. Ross¹, Ashlee N. Wood¹, Jitendra S. Kanshana¹, Gabriele Schoiswohl³, Ruya Liu⁴, Aneta Kowalski¹, Faraz A. Shah¹, Byron Chuan¹, Mahesh K. Basantani¹, Divya Gupta¹, Daniel E. Weeks^{5,6}, Stephen T. McGarvey⁷, Ryan L. Minster⁶, Jenna C. Carlson⁶, Lacey Heinsberg⁸, Nicola L. Hawley⁹, Michael J. Jurczak¹, Vijay K. Yechoor¹, Ray Lu¹⁰, Erin E. Kershaw¹

¹University of Pittsburgh, Department of Medicine

²University of Pittsburgh Organoid Research Core

³University of Graz, Department of Medicine

⁴University of Maryland, Department of Medicine

⁵University of Pittsburgh, Department of Human Genetics

⁶University of Pittsburgh, School of Public Health

⁷Brown University, School of Public Health

⁸University of Pittsburgh, School of Nursing

⁹Yale University, Department of Epidemiology

¹⁰University of Guelph, Department of Molecular and Cellular Biology

Obesity and diabetes are global health threats. A BMI-increasing variant in CREBRF [rs373863828, R457Q] protects against diabetes despite increasing obesity in humans. The mechanisms by which CREBRF and its R457Q variant mediate these effects are unknown. Since beta cell failure is the common pathway to diabetes, we tested the hypothesis that CREBRF is required for functional beta cell mass (BCM). To do this, we generated mice with global deletion of *Crebrf* (*gCrebrf*KO). We then evaluated the impact of CREBRF on mechanistic determinants of functional BCM in mice *in vivo*, islets *ex vivo*, and INS-1 832/13 cells. From these studies we found that, in contrast to human carriers of the CREBRF variant, *gCrebrf*KO mice have *impaired* fasting glucose and glucose tolerance despite *lower* total body and fat mass compared to wild-type. BCM, size, and insulin content per beta cell were decreased in *gCrebrf*KO mice. In addition, *ex vivo* glucose-stimulated insulin secretion was reduced in *gCrebrf*KO islets. Gene expression analysis of islets revealed differential expression of genes involved in proliferation, cell cycle progression, cytoskeleton, and secretion. The cell autonomous effect of *Crebrf* was confirmed by demonstrating reduced proliferation in INS-1 cells following knockdown. Overall, these data suggest that CREBRF is critical in maintaining functional BCM, and that the R457Q variant may be a gain- rather than loss-of-function with respect to energy and glucose homeostasis. *gCrebrf*KO mice exhibit a dramatic decrease in serum insulin, and our results confirm reduction of beta cell size, insulin content, and stimulated insulin secretion in *gCrebrf*KO islets. We also show the cell autonomous effect of *Crebrf* by demonstrating reduced proliferation in a beta cell line following its knockdown. CREBRF is required for glucose homeostasis despite lower body weight primarily through effects on functional BCM and regulation of key genes in beta cell proliferation and cell cycle progression.

Alterations of Functional Connectivity in Olfactory–Limbic circuits are linked to Executive Dysfunction in Adults with Congenital Heart Disease

Rozovsky, Renata¹; Ceschin, Rafael¹

¹Department of Biomedical Informatics (DBMI), University of Pittsburgh

Background. Adults with congenital heart disease (ACHD) are at heightened risk for executive-function deficits and early-onset dementia. Structural abnormalities have been documented along the olfactory pathway and within higher-order limbic regions, yet their functional relevance is unclear.

Methods. Resting-state fMRI was acquired in 112 ACHD participants (61 females; $M \pm SD = 25.0 \pm 3.3$ y) and 34 healthy controls (HC; 21 females; 24.9 ± 2.9 y). An anatomically defined bilateral olfactory seed (piriform + entorhinal cortices) and a priori limbic–prefrontal regions were examined with ROI-to-ROI and seed-to-voxel analyses, adjusting for scanner. Executive skills were quantified with the Behavior Rating Inventory of Executive Function-Adult version (BRIEF-A).

Results. Relative to HC, ACHD showed hyper-connectivity between the olfactory cortex and bilateral amygdala, hippocampus, parahippocampal gyrus, and left orbitofrontal cortex ($q < .05$, FDR). Hypo-connectivity was observed with the precuneus and calcarine cortex. Within ACHD, stronger olfactory–right-amygdala connectivity correlated with poorer BRIEF Metacognition Index and the Plan/Organize, Task Monitor, and Organization of Materials subscales ($r = .2$, permutation-corrected $p < .05$).

Conclusions. ACHD is characterized by increased olfactory-limbic coupling and reduced integration with posterior default-mode hubs, patterns linked to executive difficulties. The findings implicate sensory–limbic circuit reorganization in the neuro-behavioral sequelae of CHD and highlight potential biomarkers for early cognitive decline.

A Harmonized Visual Reading Framework for Tau PET Staging in Alzheimer's Disease

Ruppert, Emma¹; Vermeiren, Marie^{2,3,4}; Medeiros, Marina S.¹; Povala, Guilherme¹; Soares, Carolina¹; Rocha, Andreia¹; Rodrigues, Matheus S.¹; Oliveira Jr., Markley¹; Mroué, Rayan¹; Ferreira, Pamela¹; Bauer-Negrini, Guilherme¹; Lussier, Firoza¹; Amaral, Livia¹; Bellaver, Bruna¹; Tissot, Cecile^{5,6}; Masdeu, Joseph⁷; Tudorascu, Dana¹; Karikari, Thomas¹; Soleimani-Meigooni, David⁸; Fortea, Juan⁹; Lowe, Val¹⁰; Oh, Hwamee¹¹; Pascual, Belen⁷; Gordon, Brian¹²; Rosa-Neto, Pedro⁶; Baker, Suzanne⁵; Ossenkoppele, Rik⁴; van de Giessen, Elsmarieke^{2,3}; Pascoal, Tharick¹

¹Department of Psychiatry, University of Pittsburgh; ²Department of Radiology & Nuclear Medicine, Amsterdam UMC, Vrije Universiteit; ³Alzheimer Center Amsterdam, Neurology, Vrije Universiteit Amsterdam; ⁴Amsterdam Neuroscience, Brain Imaging, Amsterdam; ⁵Lawrence Berkeley National Laboratory; ⁶McGill University; ⁷Houston Methodist Research Institute; ⁸University of California San Francisco, San Francisco; ⁹Hospital de la Santa Creu y Sant Pau, Universitat Autònoma de Barcelona; ¹⁰Mayo Clinic, Rochester; ¹¹Brown University; ¹²Washington University in St. Louis

Introduction: Tau-PET can detect and stage Alzheimer disease (AD) pathology; however, current regulatory-approved visual interpretation focuses on advanced disease, limiting early-stage detection and patient stratification. This study determined whether a tracer-agnostic tau-PET visual read framework improves detection of early AD pathology and patient stratification compared with the regulatory-approved approach.

Methods: In this prospective, multicenter cohort across nine sites in North America and Europe, 681 individuals aged 50 to 90 years spanning from cognitively unimpaired (CU) to dementia underwent multi-tracer tau-PET (2-4 scans per participant) with [¹⁸F]MK-6240 (n=681), [¹⁸F]flortaucipir (n=644), [¹⁸F]RO948 (n=150), and [¹⁸F]PI-2620 (n=149), along with amyloid- β PET, MRI, and cognitive assessments. Blinded clinicians performed visual reads using a developed framework that staged tau burden into five-levels.

Results: The harmonized framework achieved high inter-rater agreement across tracers for both binary positivity and five-level classification (κ , 0.77–0.93), increasing further in the A β -positive population (κ , 0.84–0.94). Inter-tracer agreement for five-level classification ranged from good to excellent (κ , 0.73–0.88). The harmonized framework identified more individuals with early-stage tau pathology than the regulatory-approved approach among cognitively unimpaired participants (prevalence ratios, 1.49 for [¹⁸F]flortaucipir and 2.15 for [¹⁸F]MK-6240) and those with mild cognitive impairment (1.12 and 1.29), with convergence in dementia. Individuals identified exclusively by the harmonized framework had higher A β burden ($P < .001$) and plasma p-tau₂₁₇ ($P < .001$). Five-level classification showed a stepwise gradient of worsening pathophysiology ($P < .001$), with large differences between low and high tau in cognitive scores (1.4-fold), A β PET (3.2-fold), and plasma p-tau₂₁₇ (2.5-fold).

Conclusion: A five-class harmonized tau-PET visual reading demonstrated high inter-rater and inter-tracer agreement, detected more early-stage tau pathology than the regulatory-approved approach, and enabled biologically validated staging. These findings support a standardized, tracer-agnostic approach to tau-PET interpretation that improves patient identification, stratification, and evaluation for disease-modifying therapies.

Cross-platform comparison of novel Rift Valley fever virus vaccine candidates

Rush, Rachael^{1,2}; Divens, Ashley^{1,3}; Connors, Kaleigh^{1,2}; Kindsvogel, Angel^{1,3}; Benton, Alex¹; Snow, Brooke¹; Romano, Gia¹; Allen, Cadence⁴; Fatma, Farheen⁴; Price, David⁵; Leung, Daisy⁵; Amarasinghe, Gaya⁴; McElroy, Anita^{1,3}; Hartman, Amy^{1,2}

¹Center for Vaccine Research, University of Pittsburgh

²Department of Infectious Diseases and Microbiology, University of Pittsburgh

³Department of Pediatrics, Division of Pediatric Infectious Disease, University of Pittsburgh

⁴Department of Medicine, Washington University School of Medicine in St. Louis

⁵Department of Pathology and Immunology, Washington University School of Medicine in St. Louis

Rift Valley fever virus (RVFV) is an emerging arbovirus that causes severe disease in humans and livestock and poses a significant threat to global public health. Despite ongoing outbreaks and clear pandemic potential, there are currently no licensed vaccines for human use, and the correlates of protective immunity remain poorly defined. Existing vaccines, many of which consist of live-attenuated strains, present limitations including safety concerns. These limitations underscore the need for safer, more effective, and broadly applicable vaccine approaches against RVFV and other bunyaviruses. As part of the ReVAMPP R2P2 network, we aim to establish a generalizable framework for vaccine development against bunyaviruses using RVFV as a prototype pathogen. In this study, we evaluated multiple vaccine platforms targeting the RVFV glycoproteins Gn and Gc, including protein subunit formulations, nucleic acid lipid nanoparticle (LNP) constructs, and vesicular stomatitis virus (VSV)-vectored vaccines. Vaccine efficacy was assessed in a lethal murine challenge model using the wildtype pathogenic ZH501 strain of RVFV, and immune profiling was conducted to define correlates of protection. Across platforms, the tested vaccines demonstrated various levels of efficacy. VSV-vectored vaccines consistently provided robust levels of protection at a single tested dose, while nucleic acid and protein-based approaches indicated variable efficacy depending on the antigen design and dose. Across all tested platforms, vaccines elicited antigen-specific IgG responses. However, protection did not consistently correlate with total antibody or neutralizing titers. Fc effector function profiling revealed distinct functional signatures across platforms, suggesting that non-neutralizing antibody functions may contribute to protection. These findings highlight the importance of both vaccine platform and antigen design in shaping protective immunity. Collectively, this work provides a comparative framework for evaluating vaccine strategies and informs the rational design of broadly protective countermeasures against RVFV and related emerging bunyaviruses.

Mass Spectrometry Profiling of PNS-Tau Reveals Tissue-Specific Signatures in Heart and Kidney

Sanotra, Monika R.¹; Zeng, Xuemei¹; Karikari, Thomas K.¹

¹Department of Psychiatry, University of Pittsburgh

Purpose: PNS-tau differs from brain-derived tau (BD-tau) by including two extra exons (4a and 6). Co-morbid conditions like cardiovascular and kidney disease can affect the accuracy of tau-based Alzheimer's disease (AD) biomarkers, likely due to PNS-tau from peripheral tissues. To better understand PNS-tau and identify co-morbidity-specific markers that could improve AD biomarker performance, we combined in vitro studies of recombinant PNS-tau and BD-tau with in vivo profiling using co-immunoprecipitation and mass spectrometry (Co-IPMS) on heart and kidney tissues from groups with and without co-morbidities.

Methods: Recombinant human PNS-tau and BD-tau proteins were incubated at 37 °C for up to 7 days and analyzed by ELISA and shotgun mass spectrometry for degradation profile analysis. For tissue experiments, human kidney and heart samples (n = 5 per group) were cryo-ground, homogenized in T-PER buffer, and total tau was captured using Dynabeads coated with tau antibody cocktail. The samples were digested with trypsin and analyzed by high-resolution LC-MS/MS. Database searching was performed with Peaks Studio, and tau peptide areas were extracted from raw files for accurate quantification.

Results: ELISA results demonstrated a more rapid decline in signal for PNS-tau, indicating lower stability compared to BD-tau. Mass spectrometry profiles revealed potential cleavage hotspots in PNS-tau (139-164, 406-413, 414-440, 638-657), as well as evidence of isomerization (703-712), which may contribute to its accelerated instability. Preliminary analysis showed tissue-specific expression patterns: heart tissue exhibited higher levels of tau exon 4a, whereas kidney tissue displayed increased expression of exon 6. Both had low abundance of the microtubule-binding region. Co-IPMS analysis identified potential PNS-tau interactors, including monoamine oxidase B and alpha-synuclein, which may help elucidate the unique functional characteristics of PNS-tau.

Conclusion: Our study reveals key differences between PNS-tau and BD-tau and lays the groundwork for developing PNS-tau biomarkers to improve the accuracy of AD diagnosis.

Cefiderocol selection drives parallel molecular evolution with divergent outcomes in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

Satterwhite, Rebecca S.^{1,2}; Cooper, Vaughn S.¹

¹**Department of Microbiology and Molecular Genetics, University of Pittsburgh**

²**Cancer Epidemiology and Prevention Program, UPMC Hillman Cancer Center, University of Pittsburgh**

Nutritional immunity is an innate vertebrate defense strategy in which hosts attempt to starve pathogens by removing iron from the site of infection. “Trojan Horse” drugs (antibiotics with iron-binding moieties) represent a new method to enhance this natural immune response. These drugs are actively imported by the pathogen, which cannot fully disable its iron-import machinery due to the nutrient requirement. To date, only one Trojan Horse drug, cefiderocol (FDC), a cephalosporin with an iron-binding moiety, has been FDA-approved for widespread clinical use. The extent to which nutritional immunity alters the evolution of antibiotic resistance remains an open question. To address this question, we combined experimental evolution with high-resolution barcoded lineage tracking to investigate how iron availability shapes adaptation to FDC, and how concurrent nutrient and antibiotic stresses impact population genetic diversity. We evolved barcoded libraries of *Acinetobacter baumannii* strain 17978 and *Pseudomonas aeruginosa* strain MPAO1 in a host-defined nutrient medium varying in iron content and FDC presence. We found that iron limitation decelerated adaptation to FDC for *P. aeruginosa*, but not *A. baumannii*, which evolved widespread heteroresistance regardless of iron availability. Lineage tracking revealed clear, strain-specific effects of iron and antibiotic stress on population genetic diversity, while whole-genome population sequencing showed that selection repeatedly targeted similar functions in both strains. Thus, similar genetic solutions gave rise to markedly different resistance phenotypes, highlighting a decoupling between molecular evolution and clinically relevant outcomes that depends strongly on genetic background. Together, our results demonstrate that the nutrient environment fundamentally shapes the tempo, pathways, and predictability of antibiotic adaptation, and that molecular parallelism does not necessarily translate into phenotypic parallelism.

Comparative Clinical Concordance of Tau-PET Staging Frameworks Across Tracers

Scop Medeiros, Marina¹; Ruppert, Emma¹; Soares, Carolina¹; Rocha, Andreia¹; Ferreira, Pamela L.¹; Bellaver, Bruna¹; Povala, Guilherme¹; Negrini, Guilherme¹; Amaral, Livia¹; Rodrigues, Matheus¹; Oliveira, Markley Silva¹; Mroué, Rayan¹; Masdeu, Joseph²; Soleimani-Meigooni, David³; Fortea, Juan⁴; Lowe, Val⁵; Oh, Hwamee⁶; Pascual, Belen²; Gordon, Brian⁷; Rosa-Neto, Pedro⁸; Baker, Suzanne L.⁹; Pascoal, Tharick A.¹

¹University of Pittsburgh

²Houston Methodist Research Institute

³University of California San Francisco

⁴Hospital de la Santa Creu i Sant Pau

⁵Mayo Clinic

⁶Brown University

⁷Washington University in St. Louis

⁸McGill University Research Centre for Studies in Aging

⁹Lawrence Berkeley National Laboratory

Background: Tau PET staging frameworks are increasingly used to biologically define Alzheimer's disease progression. However, multiple anatomical hierarchies and thresholding strategies exist, and their clinical concordance across tracers remains unclear.

Methods: We compared tau PET staging frameworks across four factors: tracer (MK-6240 vs Flortaucipir), anatomical framework (data-driven, Braak-based, Alzheimer's Association-based, and mixed), thresholding strategy (amyloid-negative cognitively unimpaired, young reference, and Gaussian mixture), and clinical staging definitions (four variants). Analyses followed a fully crossed factorial design (992 combinations per tracer) in 281 amyloid-positive participants from the HEAD study. Clinical-biological concordance was assessed using weighted quadratic κ , with mixed-effects models used to estimate adjusted effects.

Results: Concordance varied substantially across frameworks and thresholding strategies. No single anatomical framework consistently outperformed others. MK-6240 generally showed higher concordance than Flortaucipir. Mixed-effects models identified significant effects of tracer and thresholding strategy, as well as tracer-dependent interactions, while differences across framework families were modest after adjustment. Data-driven and temporo-neocortical frameworks using Gaussian mixture thresholds showed the most consistent performance across tracers.

Conclusion: Tau PET staging performance depends strongly on tracer and thresholding strategy, with framework differences partly driven by threshold selection. These findings highlight the need for harmonized, tracer-aware evaluation and caution against assuming generalizability across tracers.

Deep Plasma Proteomics Reveals Network-Level Signatures of Astrocyte Reactivity Centered in the Precuneus

Shi, Ruyu^{1,2}; Zeng, Xuemei^{1,2,3}; Balogun, Wasiiu G.^{1,2,3}; Farinas, Marissa F.^{1,2,3}; Lopresti, Brian J.^{1,2}; Ikonomic, Milos D.^{2,4,5}; Aizenstein, Howard J.^{1,2}; Villemagne, Victor L.^{1,2}; Cohen, Ann D.^{1,2}; Karikari, Thomas K.^{1,2,3}

¹Department of Psychiatry, University of Pittsburgh

²Alzheimer's Disease Research Center, University of Pittsburgh

³Biofluid Biomarker Laboratory, Western Psychiatric Hospital, University of Pittsburgh Medical Center

⁴Department of Neurology, University of Pittsburgh

⁵Geriatric Research Education and Clinical Center, VA Pittsburgh HS

Astrocyte reactivity is an early, spatially selective feature of neurodegenerative disease, with preferential involvement of precuneus and posterior cingulate during preclinical stages. Identifying circulating proteins that reflect regional astrocyte activation could yield scalable biomarkers and improve understanding of early glial pathophysiology. PET imaging with [¹⁸F]SMBT-1, which binds monoamine oxidase-B (MAO-B) overexpressed in reactive astrocytes, enables in vivo assessment of regional astrocyte reactivity.

Eighty-five preclinical participants (51.8% female; mean age 68.1±7.4 years; 16.5% amyloid-β-positive) underwent regional SMBT-1 PET imaging and plasma proteomics. Low-abundance plasma proteins were enriched using Proteograph XT, digested with trypsin, and analyzed by Orbitrap Astral mass spectrometer coupled to a Vanquish Neo UHPLC. Protein analyses incorporated age- and sex-adjusted partial Spearman correlations, limma differential abundance analysis, and weighted gene co-expression network analysis (WGCNA) to identify proteins and modules associated with regional SMBT-1 SUVR.

In total, 94,712 peptides and 7,742 protein groups were identified, with 6,536 proteins reliably quantified by at least two peptides. Partial Spearman analysis identified 65 proteins positively associated with precuneus SMBT-1 SUVR ($p < 0.001$; FDR=8%). WGCNA identified 13 co-expression modules, of which ME2 (355 proteins) showed the strongest association ($p < 0.001$). Integrating regional association strength and network centrality, we prioritized 17 Tier 1 proteins supported by convergent evidence from partial Spearman, limma, and WGCNA, and 60 Tier 2 proteins supported by partial Spearman and network-based correlation. Gene Ontology showed enrichment for mitochondrial energy metabolism, oxidative phosphorylation, nucleotide metabolism, and acyl-CoA-related pathways. Cross-region evaluation showed that Tier 1 proteins had the strongest correlations in the precuneus, followed by posterior cingulate, supramarginal gyrus, and orbitofrontal cortex. These findings define a metabolically enriched, network-embedded plasma proteomic signature of regional astrocyte reactivity in preclinical stage.

Isolating the Vibrational Spectra of the Red Chlorophylls in Photosystem I with Multispectral Two-Dimensional Spectroscopy

Shipp, James D.¹; Li, Chenshuai²; Lee, Yumin²; Gorka, Michael³; Golbeck, John H.⁴; Anna, Jessica M.¹

¹Department of Chemistry, University of Pittsburgh

²Department of Chemistry, University of Pennsylvania

³Department of Chemistry and Chemical Biology and The Baruch '60 Center for Biochemical Solar Energy Research, Rensselaer Polytechnic Institute

⁴Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park

Cyanobacterial Photosystem I is a trimeric light harvesting complex, responsible for sunlight driven reduction of ferredoxin during photosynthesis. The function of the PSI complex relies on a charge separation process in the Reaction Center (RC) core that takes place with ~100% efficiency. Whilst the charge-separated state has been characterized on long timescales, there are unanswered questions regarding the initial steps of electron transfer in the PSI core.

Ideally, the electron transfer mechanism in the isolated PSI RC could be studied using ultrafast spectroscopy; however, it is not possible to separate the RC core from the antenna chlorophylls in PSI. Therefore, we first require detailed characterization of the PSI antenna excited states to enable studies of ultrafast electron transfer within the RC core.

While femtosecond mid-IR spectroscopy is a powerful tool for probing charge separated states, it cannot yet be effectively applied to the PSI RC core, as the IR spectrum of the excited state antenna chlorophylls is not fully understood. This is especially challenging for the 'Red Chlorophyll' sites, which absorb at lower energies compared to the P₇₀₀ special pair and form charge transfer states upon excitation. In this study, we apply two-dimensional electronic-vibrational (2DEV) spectroscopy to PSI for the first time. 2DEV is a multidimensional spectroscopy that probes the vibrational states of the antenna following broadband visible light excitation with high spectral and temporal resolution. We apply 2DEV here to characterize the initial excited states of the Red Chls, identifying the vibrational absorptions of the excited state cationic and anionic groups within their charge transfer states.

Uncovering the role of a “late bloomer” — the paralaminar amygdala nucleus

Shvedov, Naomi R.^{1,3}; Bandi, Akhil C.^{2,3}; Runyan, Caroline A.^{1,3}; Sorrells, Shawn F.^{1,3}

¹Department of Neuroscience, University of Pittsburgh

²Neuroscience Institute, Carnegie Mellon University

³Center for the Neural Basis of Cognition, University of Pittsburgh

In the amygdala, the paralaminar nucleus (PL) remains immature after birth. In humans and other mammals, the PL contains a reservoir of immature neurons that persist into adolescence and beyond. These cells express DCX, have simple morphology, and retain a capacity to migrate within PL and to nearby regions in the amygdala and cortex. When these neurons finally mature, they become excitatory neurons, integrating into circuits mediating olfaction, emotion, social behavior, and memory. The precise functional role of the late-maturing PL remains unknown, despite its clinically-relevant position within limbic circuitry implicated in neuropsychiatric illnesses.

In mice, immediate early gene experiments from our lab have suggested that PL neurons are responsive to novel and social olfactory stimuli, however the dynamics and specific tuning of PL neurons, as well as their contribution to social behavior, are unknown. To determine how PL neurons contribute to social olfactory processing, I propose a series of experiments using *in vivo* two-photon (2P) microscopy through implanted deep-brain lenses in adolescent and adult mice to visualize PL dynamics during behavior. Here I demonstrate feasibility of targeting PL, a small, deep brain nucleus (4.5 mm below dura) with GRIN lenses for *in vivo* 2P imaging, and present the first known recordings from this area. I also describe an experimental approach for testing the role of PL in social olfactory discrimination tasks in adult mice, and our plans to determine how the function of PL emerges as it matures over adolescence.

Cutaneous STING pathway agonism enhances the immunogenicity of a SARS-CoV-2 ferritin nanoparticle vaccine in both young and aged mice

Singh, Sanpreet¹; Balmert, Stephen C.¹; Mahendiratta, Saniya¹; Hajduczki, Agnes^{2,3}; Carey, Cara Donahue¹; Rees, Phyllis A.^{2,3}; Dhayani, Ashish¹; Zhang, Jiying¹; Chen, Jeffrey¹; Sumpter, Tina L.^{1,4}; Matyas, Gary R.⁵; Rao, Mangala⁵; Joyce, M. Gordon^{2,3}; Korkmaz, Emrullah^{1,6}; Falo Jr, Louis D.^{1,6}

¹Department of Dermatology, University of Pittsburgh

²Emerging Infectious Diseases Branch, Walter Reed Army Institute of Research

³Henry M. Jackson Foundation for the Advancement of Military Medicine

⁴Department of Immunology, University of Pittsburgh

⁵U.S. Military HIV Research Program, Walter Reed Army Institute of Research

⁶Department of Bioengineering, University of Pittsburgh

Low thermotolerance, waning immunity, limited breadth, and age-dependent immunogenicity of existing SARS-CoV-2 vaccines justify the development of new thermostable vaccines capable of inducing durable protection against existing and future coronaviruses across the lifespan. Here, we report a thermotolerant coronavirus vaccine candidate combining SARS-CoV-2 Spike protein Ferritin Nanoparticle (*SpFN*) and the rationally selected STING pathway agonist ADU-S100 in a microneedle patch (MNP). We demonstrate that skin delivery of SpFN+ADU-S100 MNP elicits improved humoral responses in young and aged mice compared to IM injection of monomeric SARS-CoV-2 Spike protein without any systemic reactogenicity. SpFN+ADU-S100 MNP improves antigen-specific adaptive immune responses by inducing Th1-predominant pro-inflammatory cytokines and chemokines in the vaccine-targeted skin microenvironment. Skin vaccination with SpFN+ADU-S100 MNP generates robust, durable, broadly neutralizing antibodies, and potent systemic and pulmonary cellular responses against coronaviruses. Notably, the key immunopotentiating benefits of ADU-S100 as a skin adjuvant is retained in both young and aged mice, as well as in translational studies using human skin models. Together, SpFN+ADU-S100 MNP supports the utility of emerging bioengineering platforms and adjuvants for the development of a next-generation coronavirus vaccine towards addressing the limitations of authorized SARS-CoV-2 vaccines.

Protective Effects of Supportive Adults on Mental Health and Substance Use Among Adolescents Exposed to Problematic Parental Substance Use

Smith, Brittany T.¹; Wilson, Tyia²; Szoko, Nick³; Hanner, Cassey²; Strotmeyer, Stephen⁴; Guyon-Harris, Katherine⁵; Culyba, Alison²

¹Department of Epidemiology, University of Pittsburgh

²Division of Adolescent and Young Adult Medicine, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh

³Division of Adolescent Medicine, Department of Pediatrics, Washington University

⁴Allegheny County Health Department, Pittsburgh, PA

⁵Division of General Academic Pediatrics, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh

Adverse childhood experiences (ACEs), including problematic parental substance use, put adolescents at risk for numerous negative health outcomes. The presence of a pro-social supportive adult has been found to positively impact a broad range of adolescent outcomes and mitigate the consequences of risk factors. Therefore, the purpose of this study was to 1) examine associations between parental substance use and ACE score and adolescents' mental health and substance use, and 2) explore potential direct and buffering protective effects of a supportive adult on these outcomes. Generalized linear mixed models were used to examine these relationships in a sample of 3,330 adolescents from 17 high schools in Allegheny County, PA. Adolescents exposed to parental substance use had higher mean ACE scores than their non-exposed peers (β : 1.62, CI: 1.51-1.73 $p < .001$). They were also less likely to report having a supportive adult when compared to adolescents without parental substance use exposure (AOR: .785, CI: .616-1.00, $p = .050$). Parental substance use and ACE score were directly associated with feeling sad or hopeless, non-suicidal self-harm, and considering suicide. The presence of at least one supportive adult was inversely associated with all mental health symptoms except non-suicidal self-harm. The presence of a supportive adult did not buffer the relationship between parental substance use and mental health. Parental substance use was directly associated with ever using marijuana or alcohol. ACE score was directly related to ever using all substances. The presence of at least one supportive adult was not directly associated with the substance use outcomes and did not provide a buffering effect. These findings suggest that parental substance use and ACE score may contribute to adolescents' risk for certain mental health and substance use outcomes. Interventions that help adolescents exposed to parental substance use build supportive adult connections could be beneficial to mental health.

Isolated Synaptic Degeneration Identifies a High-Risk Transitional State Across The Alzheimer's Disease Continuum

Soares, Carolina¹; Medeiros, Marina S.¹; Rocha, Andreia¹; Ruppert, Emma¹; Scarpatto Rodrigues, Matheus¹; Oliveira Jr, Markley¹; Mroue, Rayan¹; Bellaver, Bruna¹; Ferreira, Pamela L.¹; Lussier, Firoza¹; Tudorascu, Dana L.¹; Rosa-Neto, Pedro²; Pascoal, Tharick A.¹

¹Department of Psychiatry, University of Pittsburgh

²McGill University Research Centre for Studies in Aging

Synaptic and structural degeneration are partially dissociable processes that emerge at different stages of Alzheimer's disease (AD), yet current biomarker frameworks do not distinguish them. We examined whether separating these two dimensions identifies clinically meaningful heterogeneity and improves risk stratification across the AD continuum. We analyzed longitudinal data from 1,217 participants spanning the AD continuum (cognitively unimpaired [n=440], mild cognitive impairment [n=556], AD dementia [n=221]) and an independent exploratory cohort (n=139). Synaptic degeneration (S) was indexed by cerebrospinal fluid total-tau (t-tau) and classical synaptic biomarkers, consistent with evidence that t-tau correlates more closely with synaptic than with neurodegeneration biomarkers; structural neurodegeneration (N) by MRI-derived hippocampal volume, yielding four S/N profiles: S-N, S+N-, S-N+, and S+N+. We evaluated group distributions, amyloid- β burden, longitudinal transitions, cognitive performance, and derived hazard ratios and absolute risk estimates for clinical progression using Clinical Dementia Rating-sum-of-boxes [CDR-SB]. The four S/N profiles differed systematically by cognitive stage and amyloid- β burden. Isolated synaptic degeneration (S+N-) was present in 26% of participants and represented the highest-risk transitional state: 45% developed structural neurodegeneration within a median of two years, and those with S+ at baseline developed structural neurodegeneration significantly faster than those without S/N pathology. S+N- showed worse cognition and faster decline than S-N-, yet declined at less than half the rate of S+N+ (0.65 vs 1.47 points/year). Compared with S-N-, hazard ratios for cognitive decline were 3.44 (S+N-) and 6.2 (S+N+), with four-year absolute risks of 26% and 46%, versus 9% in S-N-. Synaptic degeneration added independent prognostic value beyond amyloid- β burden and structural neurodegeneration, contributing 0.40 additional CDR-SB points/year, with synergistic acceleration when both pathologies coexisted. Separating synaptic from structural neurodegeneration reveals S+N- as a clinically actionable state that improves resolution within current AD framework, with direct implications for prognostic enrichment in disease-modifying trials.

A gyroid-like structure using topology optimization considering thermal and mechanical performances

Sung, Myung Kyun¹; To, Albert¹

¹Department of Mechanical Engineering and Materials Science, University of Pittsburgh

The gyroid is one of the proven lattice structures for additive manufacturing (AM), as it improves the strength-to-weight ratio and accelerates manufacturing time. However, the gyroid is not designed for specific applications, and its optimal thickness must be determined by the user prior to manufacturing. Topology optimization (TO) has been used to identify the optimal material distribution under specified boundary conditions and constraints using the finite element method. However, TO cannot guarantee periodic boundary conditions between unit cells without connectivity information, which limits its suitability for designing AM lattice structures. This research aims to develop an optimized gyroid-like structure using multi-objective topology optimization. The topology optimization is conducted under gyroid-like boundary conditions to ensure periodic boundaries while balancing both thermal and mechanical performance objectives.

CDH1 Loss Drives Tumor Immune Microenvironment Remodeling and Metabolic Reprogramming in a Syngeneic ER+ ILC-like Model

Thale, Insa^{1,2}; John Mary, Dixcy Jaba Sheeba^{1,2}; Wang, Sarah³; Rigatti, Lora⁴; Hooda, Jagmohan^{1,2}; Lucas, Peter⁵; Tseng, George³; Lee, Adrian V.^{1,2,6,7}; Oesterreich, Steffi^{1,2,6}

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh

²Women's Cancer Research Center, UPMC Hillman Cancer Center, Magee-Women's Research Institute, University of Pittsburgh

³Department of Biostatistics, University of Pittsburgh

⁴Division of Laboratory Animal Resources, University of Pittsburgh

⁵Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine and Science

⁶Cancer Biology Program, UPMC Hillman Cancer Center

⁷Institute for Precision Medicine, University of Pittsburgh

Invasive lobular carcinoma (ILC) is the most common special histological subtype of breast cancer, accounting for 10–15% of cases. Although often initially responsive to endocrine therapy, ILC is associated with poorer long-term outcomes compared to invasive carcinoma of no special type. Over 90% of ILCs are estrogen receptor-positive (ER+) and characterized by loss of E-cadherin (CDH1), yet syngeneic models to study tumor–immune interactions in this context remain limited.

To address this, a CDH1 knockout (KO) model was generated in the ER+ SSM3 cell line, derived from STAT1-deficient mice, using CRISPR-Cas9. Comprehensive characterization was performed across 2D, ultra-low attachment (ULA), and *in vivo* conditions. CDH1 KO tumors exhibited accelerated growth and show key histological features of human ILC, including single-file growth patterns, fibroplasia, and increased collagen deposition.

Immunoprofiling revealed that CDH1 loss increased M2-like macrophage polarization, consistent with an immunosuppressive tumor immune microenvironment. Luminex analysis of ULA-conditioned media demonstrated elevated secretion of KC, LIF, and VEGF, factors associated with myeloid recruitment and immune suppression. Transcriptomic analysis further identified enrichment of TNF α /NF κ B signaling and reactive oxygen species pathways, indicating inflammatory remodeling and oxidative stress. In parallel, differential expression of lipid metabolism and antioxidant genes suggested a potential ferroptosis vulnerability, supported by increased sensitivity of CDH1 KO cells to the GPX4 inhibitor RSL3 *in vitro*.

These findings establish a novel syngeneic ER+ ILC-like model in which CDH1 loss drives immunosuppressive remodeling and altered lipid metabolism, pointing toward a potential therapeutic vulnerability to ferroptosis. Ongoing and planned studies, including VECTRA multiplex staining, immune flow cytometry, Luminex profiling of tumor lysates, lipidomics, and targeted ferroptosis assays, will further validate and extend these observations.

Introducing the Communication Technology Research Lab (CTRL)

Totoni, Samantha¹; Karimova, Altynay¹; Tzanis, Zoe¹; Davis, Seth Simon¹; Kazemi, Kiana²; Levin, Carmel¹; Li, Xinyi³; Woolley, Samuel¹

¹**Department of Communication, University of Pittsburgh**

²**Department of Computer Science, University of Pittsburgh**

³**School of Public Health, University of Pittsburgh**

The team at the University of Pittsburgh's Communication Technology Research Lab (CTRL) aims to provide a human-centric, evidence-based rationale for preserving people's democratic and human rights online. Artificial Intelligence and automation are central technologies for modern communication, and we analyze how a wide range of groups around the world use these tools to enhance the information they create and disseminate. We are particularly interested in how people use combinations of the latest generative AI tools, chatbots, and emerging media technologies in bids to amplify certain streams of content. At CTRL, we interrogate the nature and impact of internet-based propaganda and are committed to translating this work for journalists, policy makers, civil society groups, and the public. Here, I highlight examples of CTRL's ongoing work. Our focus is not limited to the groups who make and build digital propaganda campaigns and content of those campaigns. We also work alongside community partners who are on the receiving end of such efforts, co-producing content about important lessons they have learned.

Do museum specimens preserve gut microbiomes? Evidence from ethanol-preserved freshwater bivalves

Vaughn, Stephanie N.^{1,4}; Atkinson, Carla L.²; Johnson, Paul D.³; Jackson, Colin R.¹

¹Department of Biology, University of Mississippi

²Department of Biological Sciences, University of Alabama

³Alabama Department of Conservation and Natural Resources, Alabama Aquatic Biodiversity Center

⁴Department of Geology and Environmental Science, University of Pittsburgh

Freshwater mussels are important indicators of the overall health of their environment but have suffered declines that have been attributed to factors such as habitat degradation, loss of fish hosts, climate change, and excessive nutrient inputs. The loss of mussel biodiversity can negatively impact freshwater ecosystems, such that understanding the mussel gut microbiome has been identified as a priority topic for developing conservation strategies. In this study, we determined if ethanol-stored specimens of freshwater mussels could yield representative information about their gut microbiome, such that changes in the microbiome through time could potentially be determined from mussel museum collections.

A short-term preservation experiment using the invasive clam, *Corbicula fluminea*, was used to validate ethanol as a method for bivalve microbiome storage, and the gut microbiomes of nine native mussel species that had been preserved in ethanol for between 2-9 years was assessed. The gut microbiome of each bivalve was characterized using Illumina 16S rRNA gene sequencing. The bacterial communities of short-term stored *C. fluminea* guts were not significantly different based on storage method (95% ethanol or frozen at -4°C). In long-term stored mussels, there were significant differences in bacterial communities based on mussel species (PERMANOVA; $p < 0.01$, $F = 1.816$), but not between storage duration or river of parental origin. Major bacterial phyla from the guts of long-term stored museum mussels were Firmicutes, Planctomycetes, Gammaproteobacteria, Alphaproteobacteria, Actinobacteria, Verrucomicrobia, Betaproteobacteria, Bacteroidetes, Acidobacteria, and Fusobacteria. Furthermore, we identified a potentially common “core” genus of bacteria (*Romboutsia*) to all freshwater bivalve species studied.

Overall, we show that ethanol preservation is a valid storage method for bivalve specimens in terms of maintaining effective sequencing depth and richness of their gut bacterial assemblages. These findings support the potential of using ethanol-preserved museum collections to examine patterns in the gut microbiome of bivalves and other specimens over long periods.

CALPHAD-guided interlayer design for crack-free additive manufacturing of copper C18150 – Inconel 625 bimetallic structures

Wang, Liyi^{1,+}; Ladinos Pizano, Luis Fernando^{1,+}; Klecka, Michael A.²; Neely, Kelsay³; Xiong, Wei¹

¹Physical Metallurgy and Materials Design Laboratory, Department of Mechanical Engineering and Materials Science, University of Pittsburgh

²RTX Technology Research Center

³NASA Marshall Space Flight Center

⁺Those authors contribute equally to this work

Additive manufacturing (AM) of bimetallic structures combining copper alloys and Ni-based superalloys is critical for extreme environmental applications. However, interface cracking during fabrication persists due to thermophysical property mismatches. By implementing a CALPHAD-based ICME framework (CALPHAD: Calculation of Phase Diagrams; ICME: Integrated Computational Materials Engineering), we decode nonequilibrium solidification and phase stability to predict cracking susceptibility. Liquid phase separation emerges as the dominant mechanism, altering solute redistribution and thermal stress accumulation – a previously underexplored factor in bimetallic systems. Experiments using wire arc additive manufacturing (WAAM) validate model prediction: crack-free interfaces between C18150 and In625 require intermediate layers with 65 wt.% In625. This composition mitigates cracking with the lowest cracking susceptibility coefficient (CSC). Importantly, we establish a quantitative correlation between phase separation and CSC, proposing a way to analyze systems exhibiting these microstructural features. This work uses ICME methodologies by linking thermochemical modeling to process optimization, offering new principles for designing defect-resistant bimetallic components in extreme environments such as rocket engine nozzles.

Characterizing Grimace Behavior and Microglia Morphology in a Mouse Model of Chronic Alcohol Withdrawal-Induced Pain

Wherry, Jim¹; Ross, Madeleine⁴; Gil, Daniela⁴; Homanics, Gregg^{1,2,4}; Farris, Sean^{1,3,4}

¹Department of Anesthesiology & Perioperative Medicine, University of Pittsburgh

²Department of Pharmacology & Chemical Biology, University of Pittsburgh

³Department of Biomedical Informatics, University of Pittsburgh

⁴Center for Neuroscience, University of Pittsburgh

Alcohol Use Disorder (AUD) is a highly prevalent disease that causes deterioration of health and a steep socioeconomic cost. Individuals with AUD often report symptoms of chronic pain and many continue consuming alcohol to combat these symptoms, especially during withdrawal. Alcohol has deleterious effects on the neuroimmune system that cause aberrant inflammation and increased sensitivity to painful stimuli. However, the neurobiological connection between AUD and chronic pain remains ill-defined. Microglia are the primary immune cells in the central nervous system and mediate neuroinflammation associated with alcohol consumption and pain responses. We hypothesized that exposure to chronic intermittent ethanol vapor (CIEV) would lead to heightened spontaneous nociception (*i.e.* grimacing) and changes in microglial morphology indicative of a reactive phenotype. After five weeks of CIEV, PainFace software showed increased grimacing behavior in mice 24 hours into withdrawal compared to air controls, representative of chronic alcohol withdrawal-induced pain (CAWIP). Grimacing behavior was negatively correlated with territory occupied by microglia in the prefrontal cortex (PFC) indicative of increased microglial reactivity. As a positive control, a separate cohort of mice were injected with lipopolysaccharide (LPS; 1.0 mg/kg) to induce innate immune activation, and grimacing behavior was measured 24 hours post-injection. LPS treatment led to increased grimacing behavior and reductions in territory occupied by microglia compared to saline treatment; similar to mice undergoing withdrawal from CIEV. These data indicate an association between spontaneous nociception and microglial reactivity in mice undergoing withdrawal from chronic ethanol exposure and identify microglia as a possible therapeutic target for treating CAWIP.

Alternative splicing of the B-insert of the mitochondrial fission driver DRP1 is indispensable to mammalian cell health and mouse development

White, Sierra¹; Pahwa, Krisha¹; Evans, Lance¹; Javed, Zaineb¹; Cheng, Ya-Yun¹; Pan, Weihua¹; Abdelnaby, Ahmed Emam²; Benson, Cory²; Jurczak, Michael¹; Trebak, Mohamed²; Hempel, Nadine¹

¹Department of Medicine, University of Pittsburgh

²Department of Pharmacology and Chemical Biology, University of Pittsburgh

Mitochondria are essential to cell health due to their roles in a host of cellular processes. Due to their importance, mitochondria must be tightly maintained. While mitochondrial fusion is known to enhance respiration and repair of damaged mitochondria, mitochondrial fission has been shown to reduce metabolic capacity, enhance production of reactive oxygen species, and facilitate clearance of irreparably damaged mitochondrial segments through mitophagy. Maintaining a balance between the two is necessary for cell health and response to stressors. As such, disruption of these processes has been implicated in a large array of diseases. Fission disruption has been highly implicated in disease pathogenesis, particularly through alterations in expression and activity of the fission-driving GTPase DRP1. DRP1 is often modified via post-translational modifications in disease states, thus altering fission capacity. Interestingly, DRP1 is also altered via alternative splicing. 3 primary splicing sites of the transcript exist, exon 3 (A-insert), and exons 16 and 17 (B-insert). Exon 3 is spliced in most tissues. Exons 16 and 17, however, are heterogeneously spliced across different tissue types. Literature suggests absence of the B-insert enhances basal GTPase activity when compared to the isoform containing the B-insert. B-insert absence also allows DRP1 to directly interact with mitochondrial membrane without the need for mitochondrial-bound adaptors. One manuscript, however, suggested that differences induced by the B-insert were nullified in the presence of fission adaptors. To determine the biological relevance of the B-insert, we used CRISPR-Cas9 technology to generate mouse strains that exclusively express DRP1 either with or without the B-insert. Characterization of these mice as well as embryonic fibroblasts has revealed that while DRP1 lacking the B-insert does indeed tend to induce a higher amount of fission, capacity between variants does not differ. And though mitochondrial function is altered by B-insert splicing, mouse development is not impacted.

Cancer Surgery Outcomes after Implementation of a Global Budget Model in Rural Hospitals

Whitman, Jacob¹; Roberts, Eric T.²; Yabes, Jonathan G.³; Bourne, Donald¹; Sun, Zhaojun¹; Bhattacharya, Manisha³; Kahn, Jeremy M.³; Jacobs, Bruce L.³; Sabik, Lindsay M.¹

¹University of Pittsburgh, School of Public Health

²University of Pennsylvania, Perelman School of Medicine

³University of Pittsburgh, School of Medicine

This study evaluated the association between implementation of the Pennsylvania Rural Health Model (PARHM), a global budget payment program for rural hospitals, and short-term outcomes among rural patients undergoing cancer surgery. Rural hospitals face persistent financial instability that threatens access to essential services, including surgical oncology. Global budget models are designed to stabilize hospital revenue and preserve access to care, yet their impact on quality of care for complex surgical services remains uncertain. We conducted a repeated cross-sectional study using a stacked difference-in-differences design, leveraging the staggered adoption of PARHM across rural hospital service areas (HSAs) from 2019 to 2021.

Using linked Pennsylvania Health Care Cost Containment Council inpatient discharge data and Pennsylvania Cancer Registry data from 2016 to 2023, we identified patients undergoing inpatient surgery for 11 common cancer types. Patients residing in HSAs with PARHM-participating hospitals were compared to those in PARHM-eligible but non-participating HSAs using an intention-to-treat approach. Models adjusted for patient demographics, cancer characteristics, comorbidities, and area-level socioeconomic factors, and incorporated HSA and year fixed effects with propensity score weighting to account for differences in participation. The analytic sample included 17,048 surgical admissions, of which 3,537 occurred in PARHM HSAs. Across all outcomes, including 30-day and 90-day mortality, readmission, and composite measures, PARHM implementation was not associated with statistically significant changes.

Findings were consistent across adoption cohorts and cancer types, with no evidence of differential pre-trends and results robust to sensitivity analyses. These findings suggest that while PARHM may support financial stability for rural hospitals, it was not associated with measurable improvements in short-term surgical oncology outcomes during the study period.

Nasal epithelial transcriptomic profiles identify distinct asthma endotypes in a study of Puerto Rican and Dominican adults

Yang, Sheng¹; Han, Yueh-Ying¹; Pirzada, Amber²; Kaplan, Robert³; Rosser, Franziska J.¹; Forno, Erick⁴; Hudes, Golda⁵; Chen, Wei¹; Isasi, Carmen⁶; Celedón, Juan C.¹

¹Division of Pediatric Pulmonary Medicine, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh

²Institute for Minority Health Research, University of Illinois College of Medicine

³Public Health Sciences Division, Fred Hutchinson Cancer Center

⁴Division of Pulmonary, Allergy, and Sleep Medicine, Riley Children's Hospital, Indiana University School of Medicine

⁵Department of Medicine, Albert Einstein College of Medicine

⁶Department of Epidemiology and Population Health, Albert Einstein College of Medicine

BACKGROUND Categorization of asthma endotypes has traditionally relied on studying transcriptomic profiles in bronchial samples obtained during a bronchoscopy, an invasive procedure. Whether nasal epithelial transcriptomic profiles can identify asthma endotypes in adults is unclear.

OBJECTIVE To determine if nasal epithelial transcriptomic profiles can identify asthma endotypes in Latino adults, and to characterize potential risk factors for such endotypes.

DESIGN, SETTING, AND PARTICIPANTS Case-control study of asthma including 648 Puerto Rican and Dominican adults enrolled at the Bronx (NY) and Chicago (IL) sites of the Hispanic Community Health Survey/Study of Latinos (HCHS/SOL). RNA-sequencing was conducted in nasal epithelial samples from study participants. Multinomial logistic regression analysis was used for the analysis of asthma endotypes, using controls as the reference group and adjusting for age, sex, Hispanic subgroup, body mass index (BMI), birthplace in the continental U.S., smoking status and intensity, chronic obstructive pulmonary disease (COPD), and pre-bronchodilator FEV1 and FEV1/FVC.

RESULTS Using K-means clustering and Gaussian mixed modeling based on the expression of thirteen "signature genes" for T2, T17 and T1 immunity, five asthma endotypes were identified: T2^{HIGH}, T17^{HIGH}, T1^{HIGH}, T17^{HIGH}/T1^{HIGH}, and T2^{LOW}/T17^{LOW}/T1^{LOW}. Compared with controls: female sex was associated with T1^{HIGH}, T17^{HIGH}/T1^{HIGH}, and T2^{LOW}/T17^{LOW}/T1^{LOW} asthma; Puerto Rican ethnicity was associated with T17^{HIGH} asthma; current smoking was associated with T2^{LOW}/T17^{LOW}/T1^{LOW} asthma; COPD was associated with T17^{HIGH} and T17^{HIGH}/T1^{HIGH} asthma; and FEV1/FVC was lower in all endotypes except T2^{HIGH}. Pathway analysis of differentially expressed genes showed enrichment for: immune regulation processes in T2^{HIGH} asthma, neutrophil chemotaxis pathways in T17^{HIGH} asthma, T cell activation pathways in T1^{HIGH} asthma, and epidermal differentiation pathways in T2^{LOW}/T17^{LOW}/T1^{LOW} low asthma. Similar results were obtained in sensitivity analyses excluding smokers or participants with COPD.

CONCLUSIONS AND RELEVANCE Nasal epithelial transcriptomic profiling identified five asthma endotypes in Puerto Rican and Dominican adults. Replication analyses are now ongoing.

Metabolomics-guided discovery of host–microbe metabolites reveals new bile acid and tryptophan-derived signals

Ye, Yan-Song¹

¹Department of Pharmaceutical Sciences, University of Pittsburgh

The gut microbiome and host enzymes cooperatively expand the chemical diversity of small molecules, yet many host–microbe co-metabolites remain structurally and functionally uncharacterized. My research uses metabolomics-guided discovery to define new signaling molecules generated at the host–microbe interface, with a focus on bile acid and tryptophan metabolism. Using metabolomics, chemical synthesis, and functional assays, I identified previously unrecognized bile acid conjugates generated by gut microbial communities through multiplexed metabolism and interspecies cross-feeding, suggesting that microbial bile acid transformation produces a broader class of signaling molecules than previously appreciated. In parallel, I identified the murine enzyme RECON as a mammalian catalyst of C-7 oxidation of primary bile acids, challenging the long-standing view that 7-oxo bile acids are exclusively microbial in origin and demonstrating that host enzymes also directly reshape bile acid signaling pools. I am also systematically mapping gut microbial tryptophan metabolism using isotope-guided metabolite discovery, which has revealed previously uncharacterized metabolites and further supports the idea that microbial tryptophan catabolism generates novel host-relevant signaling molecules, including candidate AhR agonists. Together, these studies highlight host–microbe co-metabolism as a rich source of previously hidden chemical signals, with potential roles in intestinal barrier function, immune responses, and host physiology.

Stepwise binding of PP2A small-molecule activators alters PR65 scaffold dynamics to stimulate holoenzyme assembly

Yilmaz, Sema Z.¹; Banerjee, Anupam^{2,3}; Saha, Satyaki^{2,3}; Ohlmeyer, Michael⁴; Gordon, Reuven⁵; Itzhaki, Laura S.⁶; Bahar, Ivet^{2,3,7}; Gur, Mert¹

¹Department of Computational and Systems Biology, University of Pittsburgh

²Laufer Center for Physical and Quantitative Biology, Stony Brook University

³Department of Biochemistry and Cell Biology, Stony Brook University

⁴Atux Iskay LLC

⁵Department of Electrical and Computer Engineering, University of Victoria

⁶Department of Pharmacology, University of Cambridge

⁷Department of Chemistry, Stony Brook University

Protein phosphatase 2A (PP2A) is a heterotrimeric enzyme whose assembly and activity are orchestrated by the flexible scaffold PR65, a 15 HEAT repeat protein capable of compact and extended conformations. Small-molecule activators of PP2A (SMAPs), including DT-061 and ATUX-8385, have emerged as promising therapeutic modulators, yet the structural basis of their action on PR65 remains unresolved. Here, we combined molecular docking with extensive all-atom molecular dynamics (MD) simulations ($\sim 13 \mu\text{s}$) to map SMAP binding sites and their effects on PR65 conformational dynamics. Simulations tested two candidate sites: S1, the cryo-EM pocket for DT-061 between helices 2_i-3_i, and S2, a docking-predicted site spanning helices 4_i-5_i and 5_o-6_o. S2 proved to be a high-affinity site for both SMAPs, stabilized by Y154, R166, F191, and N199. By contrast, SMAPs bound at S1 on PR65 alone were unstable; both ligands dissociated rapidly, with DT-061 consistently relocating to a distinct site, S4 (helices 5_i-7_i), overlapping a previously identified S3 pocket in extended PR65. At S4, anchoring residues L221 and R257 stabilized DT-061 binding. These data support a sequential mechanism in which SMAPs first capture PR65 at S2 or S4, depending on conformational state, then transition through S3 into the S1 pocket that stabilizes only in the context of holoenzyme assembly. Binding at S3 or S4 biased PR65 toward extended states, favoring recruitment of catalytic and regulatory subunits. Our findings reveal how SMAPs remodel PR65 to stabilize conformations that promote trimer formation and PP2A activation, providing a structural rationale for their therapeutic mechanism.

Detecting metastatic osteosarcoma using Dinutuximab-IRDye800-DTPA in a mouse model

Yip, Catherine¹; Pant, Arjun¹; Rosenblum, Lauren¹; Chagantipathi, Bhuvitha²; Kohanbash, Gary²; Malek, Marcus³

¹Department of Surgery, University of Pittsburgh

²Department of Neurological Surgery, University of Pittsburgh

³Division of Pediatric General and Thoracic Surgery, UPMC Children's Hospital of Pittsburgh

Introduction: Osteosarcoma is the most common pediatric primary bone malignancy, frequently diagnosed at advanced stages with metastatic disease. Obtaining adequate margins at the primary resection site and pulmonary metastasectomy have demonstrated survival benefit. However, lesion localization during metastasectomy is complicated by varying size and depth of lesions, and difficulty correlating palpated lesions to preoperative imaging. As GD2 is overexpressed in osteosarcoma, we sought to utilize Dinutuximab, a chimeric antibody targeting GD2, conjugated to a fluorophore, IRDye800, and DTPA, a radioisotope chelator, to enhance intraoperative imaging in a murine model of metastatic osteosarcoma.

Methods: Human 143B cells were injected intratibially into 6-week-old NSG mice (n=4) to generate metastatic osteosarcoma. Tumors were grown for 4.5 weeks, then Dinutuximab-IRDye800-DTPA administered via tail vein injection. 4 days later, mice were euthanized, and fluorescent biodistribution studies performed. Mean fluorescence of resected tumor and organs were analyzed with one-way ANOVA.

Results: Orthotopic osteosarcoma xenografts with grossly visible pulmonary and hepatic metastases grew in 4/4 mice. Four days after tracer injection, fluorescent biodistribution studies demonstrated tracer specificity for the tumor ($37.81 \text{ AU/p2} \pm 8.63$) compared to other organs, including blood ($2.36 \text{ AU/p2} \pm 3.06$, $p < .005$) and ipsilateral muscle ($1.102 \text{ AU/p2} \pm 0.52$, $p < .001$).

Conclusions: In this study, we demonstrate specific accumulation of Dinutuximab-IRDye800-DTPA in a murine model of osteosarcoma. This suggests that a Dinutuximab-based imaging agent can be applied to the surgical management of osteosarcoma, both in resection of the primary tumor and localizing metastatic disease. Future studies will determine the depth and size limitations of Dinutuximab-IRDye800-DTPA.

Bidirectional gut microbial metabolism of dietary N-acyl amides shapes host CysLTR2 signalling

Yu, Wen-chao^{1,*}; Jian, Wenya^{2,3,*}; Song, Deguang⁴; Peng, Zhiyuan¹; Zhao, Yanyu^{2,3}; Godugu, Bhaskar⁵; Hu, Wei^{3,4}; Palm, Noah W.⁴; Crawford, Jason M.^{2,3,5}; Wu, Qihao^{1,4,†}

¹Department of Pharmaceutical Sciences, University of Pittsburgh

²Department of Chemistry, Yale University

³Institute of Biomolecular Design and Discovery, Yale University

⁴Department of Immunobiology, Yale University School of Medicine

⁵Department of Chemistry, University of Pittsburgh

⁶Department of Microbial Pathogenesis, Yale University School of Medicine

*These authors contributed equally

†Lead contact, Correspondence: qi153@pitt.edu

Diet-derived molecules are increasingly recognized as substrates for gut microbial metabolism, yet the mechanisms and host consequences of these transformations remain poorly defined. Here, we identify a class of dietary N-acyl amides that is bidirectionally transformed by the human gut microbiota, with hydrolysis as the favored reaction. We first confirmed the structures of these metabolites and systematically mapped related analogs in public datasets, revealing that this chemical class is widespread and undergoes diverse microbiome-dependent conversions. We then identified the gut bacterium responsible for this activity, defined the underlying enzyme through *in vitro* biotransformation assays, and mapped homologous genes in metagenomic datasets, establishing the genetic basis and potential prevalence of this pathway in human gut communities. Functional profiling further showed that members of this N-acyl amide class act as agonists of the host G protein-coupled receptor CysLTR2. Together, these findings uncover a diet-microbiome-host signalling pathway in which gut microbial metabolism reshapes a class of dietary lipids with host receptor activity, expanding the molecular framework through which the microbiome modulates chemical communication at the host interface.

Galectin3 condensates repair lysosomes

Yu, Xiaojie¹; Tan, Jay Xiaojun²

¹Aging Institute, University of Pittsburgh School of Medicine and UPMC

²Department of Cell Biology, University of Pittsburgh School of Medicine

Maintaining lysosomes integrity is essential for cellular homeostasis, as lysosomal membrane permeabilization (LMP) triggers inflammatory signaling and cell death. While Galectin-3 (Gal3) is a gold-standard marker for damaged lysosomes, its acute functional role following recruitment remains elusive. Here we identify Gal3 phase separation as a primary, autonomous lysosomal repair mechanism that operates independently of established pathways, such as ESCRT and PITT. Upon lysosomal damage, glycan binding triggers rapid Gal3 recruitment and further reach to the critical concentration leading to liquid-liquid phase separation (LLPS) at the damage site, a process driven by multivalent interactions within its N-terminal intrinsically disordered region (IDR). Notably, the propensity for Gal3 phase separation directly dictates repair kinetics. We show that this phase transition is both necessary for membrane restoration *in vivo* and sufficient to reseal lysosomes *in vitro*. Furthermore, Gal3-driven lysosomal repair promotes glioblastoma (GBM) progression, and its disruption significantly sensitizes GBM cells to Temozolomide. Our findings establish phase separation as a fundamental organizing principle for organelle quality control and a critical mechanism for maintaining lysosomal membrane integrity in health and disease.

Active, Virtual, And Transient Avant-garde Resistance (AVATAR): Transforming Power Transformers into AVATAR Transformers

Zhu, Jinli¹; Do, Phan K.¹; Pourmirasghariyan, Sajed¹; Li, Yuan¹; Grainger, Brandon M.¹; Peng, Fang Z.¹

¹Department of Electrical and Computer Engineering, University of Pittsburgh

Power transformers are indispensable in modern power systems, yet their inherent inductive nature introduces reactive power, transient oscillations, and resonance, which become increasingly problematic in grids with high penetration of inverter-based resources. This work proposes Active, Virtual, And Transient Avant-garde Resistance (AVATAR), a new concept that transforms conventional power transformers into AVATAR transformers with controllable virtual-resistive characteristics. By integrating fractionally rated controllable voltage sources with the transformer, the proposed approach actively compensates inductive voltage drops and reshapes the transformer behavior from inductive to resistive without significant energy dissipation. A modeling framework is developed to describe this transformation and to explain how the proposed control achieves effective damping while maintaining efficient power transfer.

Simulation studies under load-side capacitor bank switching conditions show that the AVATAR transformer effectively suppresses voltage and current overshoot, mitigates oscillations, and eliminates reactive power flow from the grid. In the studied case, the additional converter capacity required is only about 2% of the transmission power rating, demonstrating strong practical potential. These results indicate that the proposed AVATAR concept offers a scalable and cost-effective alternative to existing damping, hybrid-transformer, and solid-state-transformer solutions for improving stability, power quality, and resilience in future inverter-dominated energy systems. A 25-kVA, 8-kV medium-voltage laboratory AVATAR transformer prototype is currently under development to validate the concept experimentally and lay the foundation for industrial applications.

Hypoxia–Macrophage–WNT5A Signaling Drives Mesenchymal Transition and Therapy Resistance in Glioblastoma

Zou, Han¹; Hu, Baoli¹

¹Department of Neurological Surgery, University of Pittsburgh

Introduction / Purpose

Glioblastoma (GBM) is the most lethal primary malignant brain tumor in adults and is characterized by rapid recurrence despite surgical resection and chemoradiotherapy. Therapeutic failure is driven in part by glioma stem cell (GSC) plasticity and microenvironmental influences. However, the mechanisms linking hypoxia, immune infiltration, and subtype transition remain incompletely defined. We investigated whether microenvironmentally induced WNT5A signaling promotes GBM subtype plasticity and therapy resistance.

Methods

We combined genetic perturbation of WNT5A in patient-derived GSCs with single-cell RNA sequencing, spatial transcriptomics, ATAC-seq, hypoxia assays in primary macrophages and endothelial cells, and functional analyses of proliferation, invasion, and response to temozolomide and radiation.

Results

WNT5A was sufficient to shift GSCs along the classical–proneural–mesenchymal continuum, promoting a slow-cycling, invasive, and chemoradiotherapy-resistant phenotype. Spatial profiling localized WNT5A expression to hypoxic, perinecrotic, and hypervascular niches enriched for tumor-associated macrophages. Macrophages exhibited the highest WNT5A expression among tumor microenvironmental cells, and hypoxia induced time-dependent upregulation of WNT5A in macrophages and endothelial cells. Epigenomic analysis revealed that WNT5A-mediated transitions resembled neurodevelopmental chromatin dynamics, coupling inflammatory NF- κ B signaling with lineage-associated chromatin remodeling. Clinically, elevated WNT5A expression in primary tumors correlated with reduced progression-free survival.

Conclusion

These findings define a hypoxia–macrophage–WNT5A paracrine loop that drives mesenchymal transition and therapy resistance in GBM. Targeting microenvironment-induced subtype plasticity may represent a rational strategy to enhance treatment durability.

Sex-Biased Erythrocyte-Microglia-Neural Progenitor Cell Interaction Drives Male Bias in Medulloblastoma Incidence and Prognosis

Zou, Han¹; Patel, Yash¹; Hu, Baoli¹

¹Department of Neurological Surgery, University of Pittsburgh

Introduction / Purpose

Medulloblastoma, the most common malignant pediatric brain tumor, exhibits a consistent male predominance in incidence and worse prognosis in boys. The biological basis of this sex disparity remains unclear. We hypothesized that sex-specific differences in neural progenitor cell (NPC) maturation, regulated by microglia, contribute to male-biased tumor susceptibility.

Methods

We analyzed human datasets to confirm sex differences in prevalence and outcome. A genetically engineered mouse model recapitulating male-biased tumor incidence was developed. NPC maturation states were assessed using developmental markers and stemness profiling. Microglial gene expression was evaluated by transcriptomic analysis. Functional studies included conditional deletion of ABCA1, modulation of BLVRB, and pharmacologic elevation of bilirubin using phenylhydrazine (PHZ). Tumor formation was evaluated in the presence of oncogenic activation.

Results

Males demonstrated increased tumor incidence and reduced survival in both human cohorts and mouse models. Male NPCs exhibited delayed maturation and increased stem-like properties compared to females at equivalent developmental stages. Female microglia expressed higher levels of ABCA1, promoting lipid transport and NPC differentiation. In contrast, male microglia showed elevated BLVRB, increasing bilirubin production. Genetic or pharmacologic elevation of bilirubin impaired NPC differentiation and significantly enhanced tumor formation under oncogenic conditions. Loss of ABCA1 similarly increased stemness and tumor susceptibility.

Conclusion

Male-biased medulloblastoma arises from delayed NPC differentiation driven by sex-specific microglial signaling. Differential regulation of lipid metabolism and bilirubin production via ABCA1 and BLVRB establishes a developmental window of heightened oncogenic vulnerability in males. These findings identify microglial metabolic pathways as potential targets for reducing sex disparities in pediatric brain tumors.

Program Sponsors

The UPPDA would like to thank
the following co-sponsors:

Office of Academic Career
Development, Health Sciences
oacd.pitt.edu

Office of the Provost
provost.pitt.edu