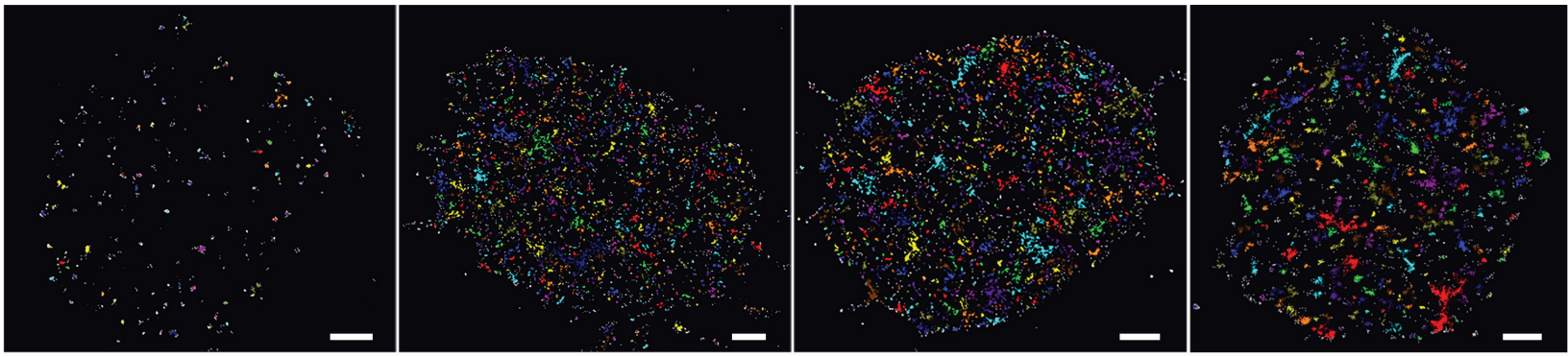
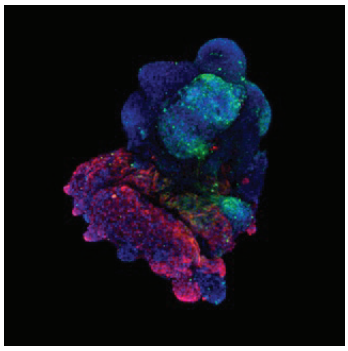
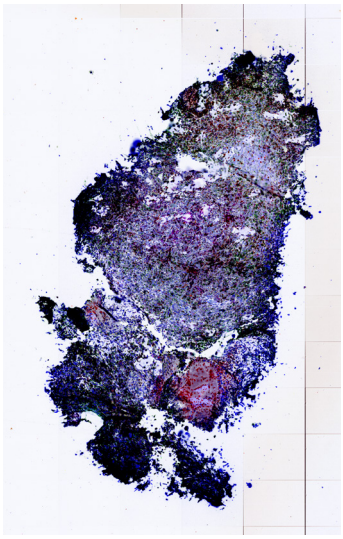
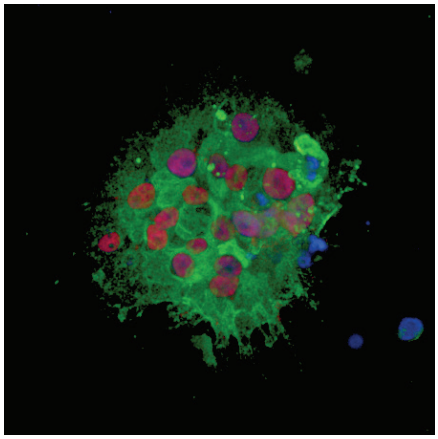


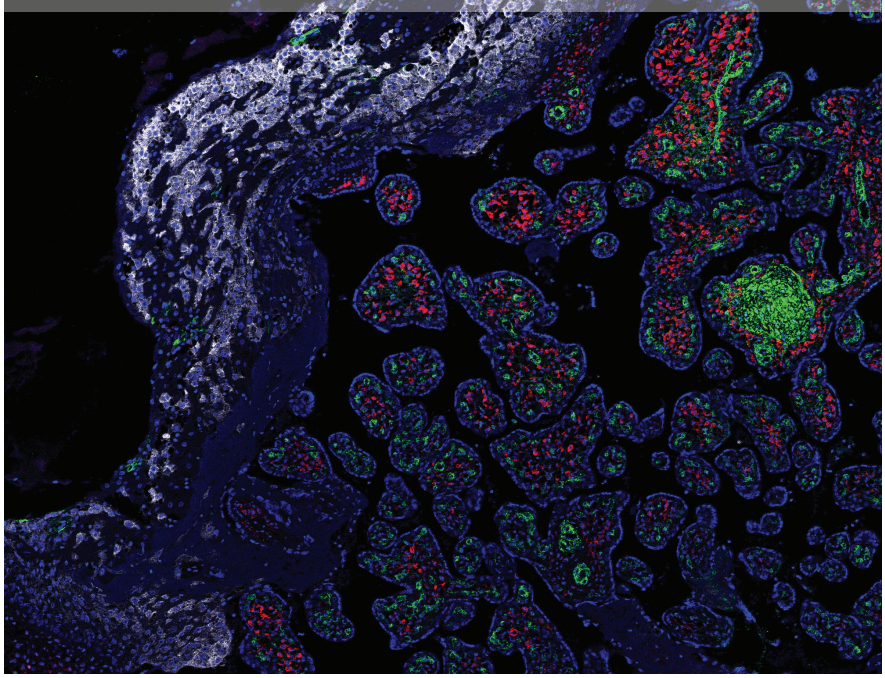






The Seventh Annual

Postdoc Research Day



September 20th
2023

The Jackson Laboratory
Leading the search for tomorrow's cures

UCONN HEALTH

Welcome to the seventh annual UConn Health/Jackson Laboratory Postdoc Research Day. I am so pleased we are returning to our full program including an exciting keynote talk from Dr. Thomas Gonatopoulos-Pournatzis from the National Cancer Institute. Most importantly, I am delighted that we are all together to celebrate our outstanding UConn Health and Jackson Laboratory postdocs. Today, we will showcase some of the important work they are performing through a series of short Speak4Science talks and a full poster session. This year, we are setting records for the most presentations at a Postdoc Research Day! On the following pages, you will find a schedule as well as abstracts for all our presenters.

I would like to thank the Health Center Research Advisory Council for their support of this year's event as well as Stephanie Holden for her help in putting this all together. We are also pleased to have support from the Jackson Laboratory for Genomic Medicine and thank Sarah Wojiski, Dawn Traficante and Rowena Grainger from Jax for all their help. Finally, I would like to thank our PDRD Planning Committee:

Sama Abdulmalik (UConn Health)
Alexander Calderon (The Jackson Laboratory)
Puja Kumari (UConn Health)
Yi Juin Liew (The Jackson Laboratory)
Roberto Vazquez Munoz (UConn Health)

Thank you for joining us in this celebration of our postdoctoral fellows during National Postdoc Appreciation Week.

Be well,

Christopher D. Heinen, Ph.D.
Director of Postdoctoral Affairs
UConn Health

Cover photo credits: *Yuanyuan Wu, Sungryong Oh, Aaron Taylor, Ramalakshmi Ramasamy*

The Seventh

Postdoc Research Day

Wednesday, September 20th, 2023

1:00	**Opening Remarks**	**Academic Rotunda**
1:05	**Speak4Science Part I**	**Academic Rotunda**
2:05	**Coffee Break**	**Rotunda Lobby**
2:20	**Speak4Science Part II**	**Academic Rotunda**
3:20	**Keynote Address:**	**Academic Rotunda**

Thomas Gonatopoulos-Pournatzis, Ph.D.

NIH Stadtman Investigator | NIH Distinguished Scholar

Head, Functional Transcriptomics Section

RNA Biology Laboratory, National Cancer Institute

Towards the Systematic Exploration of the Functional Impact of Alternative Splicing

4:30	**Poster Session and Reception**	**Rotunda Lobby**
4:40	**Poster Presentations I (Odd numbers)**	**Rotunda Lobby**
5:20	**Poster Presentations II (Even numbers)**	**Rotunda Lobby**
6:00	**End**	

Our Speak4Science event will feature a series of 4-minute talks by our postdocs. Each speaker will use one slide to broadly introduce their area of research and why it excites them. To learn more about the details of their research, I encourage you to visit the poster sessions starting at 4:30 PM. The speaker roster and their corresponding poster numbers are listed below.

Name	Title	Affiliation	Poster
1. Zeynep Altunay	*C1ql1, OPCs, and myelination*	UCH	1
2. Erica Lorenzo	*Senescence biomarkers in major depressive disorder*	UCH	3
3. Ramalakshmi Ramasamy	*Cellular atlas of senescence in placenta*	JAX	4
4. Abhay Anand	*Effects of transportation choices*	UCH	5
5. Liv Dedon	*Gut microbiome and drinking reduction*	UCH	6
6. Jessica Flori	*Defining sober harms*	UCH	7
7. Elise Bragard	*Friendship stress and drinking to cope*	UCH	8
8. Chegnliang Wang	*Regulating GSDMB pore formation: To ignite or inhibit?*	UCH	9
9. Sonia Shivcharan	*Epithelial cell sensing of intracellular bacterial invasion*	UCH	10
10. Puja Kumari	*Unveiling the immune surveillance role of EVs*	UCH	11
11. Ayano Hatori	*The effects of AMPK inhibition on CMD*	UCH	12
12. Francis O'Neill	*Novel proteins in osteosarcoma tumors*	JAX	13
13. Suranji Wijekoon	*Nanofibrous scaffolds for healing critical bone defects*	UCH	14
14. Chinedu Ude	*ACL reconstruction with tiger graft*	UCH	15
15. Aaron Taylor	*Immunosuppression in osteosarcoma tumors*	JAX	16
16. Maeva Devoucoux	*Epigenetics and splicing of aging breast*	JAX	19
17. Kevin Child	*Splicing regulation in heart development*	UCH	20
18. Amit Gupta	*Cis-elements and translation regulation*	UCH	21
19. Ashok Cheemala	*TDP-43 effects on BBB and neurodegeneration*	UCH	23
20. Sungryong Oh	*Study of human biased heart development*	UCH	24
21. Elham Ahmadi	*Trivalent oncolytic viral therapy*	UCH	27
22. Samaneh Poursaeid	*Fate determination within the stem cell niche*	UCH	28
23. Yuanyuan Wu	*Kindlin-3 regulation of integrin clustering*	UCH	29
24. Alsu Ramazanova	*EPO receptor-specific effects in MEP*	UCH	30
25. Tingting Geng	*An essential role of UBXN3B in B cell survival*	UCH	31
26. Everton Bettin	*OmpA chimeras displaying* T. pallidum *ECLs*	UCH	34
27. Sathyabaarathi Ravichandran	*CD16+ NKs impact Prevnar responses in elderly*	JAX	35
28. Atri Ta	*Immunomodulation by EHEC autotransporter*	UCH	36

Keynote Presentation

Thomas Gonatopoulos-Pournatzis, Ph.D.

Towards the Systematic Exploration of the Functional Impact of Alternative Splicing

Dr. Gonatopoulos-Pournatzis obtained his Bachelor's degree in Biology from the National & Kapodistrian University of Athens, Greece in 2007 before moving to King's College London in England to earn his Master's degree in 2008. In 2013, Dr. Gonatopoulos-Pournatzis obtained his Ph.D. from the University of Dundee in Scotland where he worked in Prof. Victoria Cowling's laboratory studying mechanisms underlying mRNA cap methylation and gene expression. For his postdoctoral research, he joined Prof. Ben Blencowe's laboratory at the University of Toronto in Canada to study the regulation and function of splicing regulatory networks in neurons with the support of postdoctoral fellowships from the European Molecular Biology Organization (EMBO), Canadian Institutes of Health Research (CIHR) and Ontario Institute of Regenerative Medicine (OIRM). Dr. Gonatopoulos-Pournatzis has received multiple awards and recognitions, including the Baxter Prize (2012), the Donnelly Centre Research Excellence Award (2018) and his is also a recipient of the 2020 NIH Distinguished Scholar Program. In 2020, Dr. Gonatopoulos-Pournatzis joined the RNA Biology Laboratory at the National Cancer Institute to establish the Functional Transcriptomics Section, which integrates functional genomics and RNA biology. His lab focuses on developing and applying CRISPR tools to systematically uncover transcript variants that play critical roles in normal physiology and disease state.

Abstracts

1. Zeynep Altunay

C1ql1 is expressed by oligodendrocyte progenitor cells and promotes recovery after demyelination.
Zeynep M. Altunay[1], Joyshree Biswas[1], Robert S. Pijewski[1], Lyndsay C. Kresic[1], Andrew Tang[1], Alexander D. Schouw[1], Akiko Nishiyama[2], Steve J. Crocker[1], David C. Martinelli[1]*

[1]Department of Neuroscience, UConn Health, [2]Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT

The central nervous system's (CNS) axon myelination is necessary for the CNS to operate properly. Oligodendrocytes which are responsible for the myelination of the CNS, originate from oligodendrocyte progenitor cells (OPCs). C1QLs bind to adhesion GPCR-B3 (ADGRB3). However, the oligodendrocyte lineage has not yet been investigated in the context of C1QL1 or ADGRB3, which inspired us to investigate whether C1QL1 has a function in the regulation of OPC maturation. We found that *C1ql1* was detected in the oligodendrocyte lineage, in both the gray matter and the white matter. We show that the gene *C1ql1* is expressed by OPCs, and not neurons, in most regions of the brain. To study the function of *C1ql1* in OPCs, we created conditional knockout mice (cKO). To induce demyelination, 8-week-old mice were fed a diet of ground standard mouse chow mixed with cuprizone powder, for 5 weeks. Following this period, mice were perfused or allowed to recover on a normal diet for an additional 2 weeks prior to perfusion. We evaluated the histology of control and cKO mice to examine if C1QL1 would have a function in regulating the propensity of an OPC to differentiate into a mature oligodendrocyte. We found that the cKO mice had a significant decrease in the density of oligodendrocytes in both the white and gray matter after the cuprizone recovery period. In this research, we demonstrate clearly that C1ql1 is expressed by OPCs. Additionally, our findings revealed that OPCs are the only cell type that expresses C1ql1 in most brain areas. With the use of cKO mice, we demonstrate that C1ql1 KO mice have a developmental delay in oligodendrocyte cell density and a reduced or slowed remyelination after a demyelination insult. Our results suggest that the C1QL1-ADGRB3 signaling pathway may have therapeutic potential for treating demyelinating diseases such as MS.

2. Ivanova Violetta
Ampyra (drug used to alleviate motor symptoms of multiple sclerosis) Potently Modulates Ethanol-Induced Effects on the Neuronal Spontaneous Electrical Activity
Violetta O Ivanova, Brianna L. Barbeau, Mei Hong Zhu, Katarina D. Milicevic and Srdjan D. Antic

Department of Neuroscience, UConn Health

Significant research effort is invested in finding additional chronic pharmacotherapy that would promote reductions in alcohol (Ethanol) intake. However, a pharmacotherapy for treating acutely intoxicated patients (for example, young people after party) and saving their lives in the emergency room is currently missing. Keeping such patients awake, agile and respiratory-compensated with a help of adjuvant drugs may be useful. We found an alcohol-induced depression of synaptically evoked population voltage (network) responses in all layers of the mouse frontal and parietal cerebral cortex; this depression can be reversed by application of 4-aminopyridine aka dalfampridine (Ampyra™). Ampyra is a "*neuron excitability booster*" used to improve walking in patients with multiple sclerosis. In our hands, in brain slices pretreated with debilitating concentrations of Ethanol (20 mM), bath application of Ampyra restores the amplitude and propagation of evoked synaptic depolarizations. The aforementioned experiments have addressed the synaptic transmission specifically, because our population voltage signals are dominated by EPSPs occurring in massive dendritic

trees of cortical pyramidal neurons, while the neuronal action potentials (spikes) are filtered out (do not contribute to the optical signals significantly). To address neuronal spiking specifically, we investigated the effects of Ethanol and Ampyra on cultured mouse neurons. We found that at a concentration of 20 mM, Ethanol caused a significant increase in the amount of spontaneous neuronal activity (spiking), while at a higher concentration of 40 mM, it actually decreased the number of spikes in the neuronal culture. Interestingly, in the presence of either 20 mM or 40 mM of Ethanol, bath application of Ampyra was able to increase the spiking frequency of the cultured cortical neurons. Our findings suggest that Ampyra is a potent modulator of Ethanol-induced changes in the cerebral cortex. Even in the presence of Ethanol-induced increase in spontaneous spiking, Ampyra was able to further increase the spiking frequencies of the neurons. Notably, at 20 mM concentration, Ethanol decreases the amplitude of EPSPs in brain slice preparations but increases the spiking of neurons in cell culture. Overall, our results indicate that Ampyra could be a promising treatment for acute alcohol intoxication, as it may help alleviate the suppressive effect of alcohol on neuronal functions (EPSPs and spiking).

3. Erica Lorenzo

Elevated burden of senescence biomarkers in immune cells of older adults with major depressive disorder

Erica C. Lorenzo[1], Jovany E. Figueroa[1,2], Ferris El-Tayyeb[1], Derya A. Demirci[1], Billy J. Huggins[1], Medha Illindala[1], Jenna M. Bartley[1], Laura Haynes[1], Breno S. Diniz[1]

[1]UConn Health Center on Aging, University of Connecticut School of Medicine, [2]Ponce Health Sciences University School of Medicine, Ponce, PR

Background: Aging is associated with the accumulation of senescent cells that can contribute to the pathophysiology of disease. Little is known about the prevalence of cellular senescence among immune cells (i.e. immune cells that express senescence markers, iSCs) nor is there a gold-standard to efficiently measure iSCs in the context of disease in humans. Major depressive disorder (MDD) in older adults has been associated with many hallmarks of senescence in whole blood, leukocytes, and plasma, supporting a strong connection between iSCs and MDD. In this preliminary analysis, we investigated the prevalence and phenotype of iSCs in older adults with MDD. Using a single-cell phenotypic approach, circulating CD4 T cells, CD8 T cells, and monocytes were examined to determine biomarkers of iSCs and correlation with severity of depression. We also assessed PBMCs for mRNA expression level of several cytokine factors known to be dysregulated with age and disease and their correlation to iSC phenotypes.

Results: Older adults with MDD (aged 69.75 ± 5.23 years) and healthy controls (aged 71.25 ± 8.8 years) were included in this study and underwent psychiatric evaluations to determine severity of depression. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood collected at study visit and examined for immune cell subset distribution and senescence biomarkers (i.e. lack of proliferation, senescence-associated heterochromatin foci (SAHF), and DNA damage). Biomarkers were assessed simultaneously in each cell and dual-expression level of SAHF and DNA damage was categorized by low, intermediate, and high expression. There was no significant correlation between high expression of biomarkers and MDD severity. A significant increase in the number of high expressing total PBMCs ($p=0.01$), monocytes ($p=0.008$), a trending increase in the number of high expressing CD4 T cells ($p=0.06$) and a higher proportion of high expressing monocytes ($p=0.05$) was observed overall in those with MDD compared to control individuals. There was also a significantly lower proportion of intermediate expressing cells in monocyte and CD4 populations in MDD compared to controls ($p=.01$ and $p=.05$, respectively). Additionally, there was a significantly higher fold change of IL-2 ($p=.0009$), IL-8 ($p=.0098$), and IL-17α ($p=.012$) mRNA expression in PBMCs with MDD and moderate to strong associations between IL-1β, IL-2, IL-6, IL-8, and TNFα and various iSC phenotypes of PBMCs in both MDD and control groups.

Conclusions: MDD is associated with increased senescence biomarkers in immune cells, where SAHF and DNA damage can be categorized by expression level. Inflammatory cytokines known to be dysregulated in plasma with age and disease show alterations with MDD and even further, within various iSC phenotypes. This research has strong clinical implications in the context of senescent biomarkers in immune cells and in assessing the contribution of iSCs to pathophysiology of disease.

4. Ramalaskshmi Ramasamy

Cellular Atlas of Senescence in Placenta

Ramalakshmi Ramasamy[1], Katherine Copeland[3], Santhosh Sivajothi[4], Juliana Alcoforado Diniz[1], Ron Korstanje[3],George Kuchel[2], Paul Robson[1]

[1]The Jackson Laboratory for Genomic Medicine, [2]Center on Aging, University of Connecticut Health Center, [3]The Jackson Laboratory, Bar Harbor, MN, [4]Single Cell Biology Laboratory, The Jackson Laboratory for Genomic Medicine

Developmental senescence (DS), known to occur in the placenta and the embryo, is a transiently programmed cellular senescence required for normal mammalian embryonic development. The evolutionary origins of deleterious senescence in aging have been proposed to result from antagonistic pleiotropy. Accordingly, since programmed DS increases the odds of successful reproduction early in life, this phenomenon is naturally selected in evolution despite its harmful antagonistic effects later in life. The placenta is a transient organ with a defined lifespan and purpose to supply the fetus with oxygen and nutrients. Classic hallmarks of cellular senescence have been described in specialized cells of the placenta called trophoblasts during placental development, as well as at the fetal-maternal interface to promote placental aging and parturition at term. Conversely, the lack of senescence in the developing placenta and during parturition results in adverse pregnancy outcomes, such as preeclampsia and fetal growth restriction. As part of the KAPP-Sen Tissue Mapping Center Collaborative (for human) and the Jax-Sen initiative (for mouse), we aim to create cellular atlases of the human and mouse placenta and map senescence in them. We will obtain human term placenta and umbilical cord from C-section delivery donors from two different age groups (young, mid-age) and mouse placenta from different embryonic time-points (E8.5, E13.5, E18.5). We use scRNA-sequencing, snRNA-sequencing, bulk RNA-sequencing, and Visium spatial transcriptomics, all unbiased approaches, to discover and characterize cell-types and cell-type-specific genes in the placenta. This information informs the design of gene and antibody panels, respectively, for Xenium *in situ* RNA and CODEX multiplex antibody-based imaging that will be employed to comprehensively characterize molecular and spatial organization of senescence cells at sub-cellular resolution in the healthy human and mouse placenta. I will present data from this work in progress. By utilizing this comprehensive suite of tissue mapping tools we will identify and extensively characterize salient features of developmental senescence and provide valuable insight into their extrapolations on age-associated cellular senescence.

5. Abhay Anand

Effects of transportation choices on personal exposure to Nitrogen dioxide (NO_2) and Noise

Abhay Anand [1], Misti Levy Zamora [1]

[1]Department of Public Health Sciences, UConn School of Medicine, UConn Health

Recent studies have reported that short-term exposures to air pollution and noise pollution can lead to negative health effects in both health-compromised and healthy individuals. Traffic exposures contribute significantly to outdoor air pollution-related mortalities. Hence, there is a need to identify effective modifiable factors/personal choices that can reduce air pollution and noise pollution exposures and evaluate their impact

on health. As part of the Effects of Transportation Choices on Commuter Health (ETCH) Study, 65 participants from Hartford County, CT are being recruited and asked to commute to work during the morning rush hour for two consecutive days and then outside peak traffic times for another two consecutive days. During the two 48-hour sampling periods for each participant, real-time $PM_{2.5}$, CO, CO_2, NO_2, O_3, and noise and cumulative NO_2 and BTEX are measured. The personal air and noise pollution exposure measurements are complemented with health assessments of blood pressure, lung function, and pulmonary inflammation of the lower airways. This presentation will focus on the relationship between commuting choices and personal exposure to NO_2 and Noise. Preliminary results show that the 48-hour averaged personal NO_2 exposures were higher during rush hour commute days (18.9 ± 32.6 ppb) as compared to non-rush hour commute days (12.0 ± 25.2 ppb) for 17 participants ($p<0.20$). The personal noise exposures were found to be highest in the commute microenvironments (median of average noise levels= 70.0 dB; Inter quartile range= 4.1 dB) compared to other microenvironments (median of average noise levels = 62.9 dB; Inter quartile range= 2.7 dB). However, the 48-hour averaged personal noise exposures did not exhibit significant variation between rush hour commute days (average noise levels ranging from 57.0 dB to 63.7 dB) and non-rush hour commute days (average noise levels ranging from 58.4 dB to 64.7 dB).

6. Liv Dedon

Gut microbiome composition influences intervention outcomes in heavy drinkers with alcohol use disorder

Dedon, L.R.[1,2], Yuan, H.[2], Arias, A.J.[3], Covault, J.M.[4], Zhou, Y.[2]

[1]Calhoun Cardiology Center, UConn School of Medicine, [2]Department of Medicine, UConn School of Medicine, [3]Department of Psychiatry, Virginia Commonwealth University School of Medicine, Richmond, VA, [4]Department of Psychiatry, UConn School of Medicine

Background: Development and severity of alcohol use disorder (AUD) has been linked to variations in gut microbiota and their associated metabolites through the gut-brain axis. Reduction in alcohol consumption has been related to microbiome composition. We show that intervention outcome is significantly correlated with baseline microbiome independent of treatment.

Methods: Stool samples (48) from a single site of a multi-site 16-week double-blind, placebo-controlled trial of Zonisamide, an FDA approved anticonvulsant shown to reduce drinking in individuals with AUD. Stools were collected and GGT and PEth levels were measured both at screening (baseline) and trial completion along with drinking behavior throughout the intervention. Stool microbial composition was analyzed *via* 16S rRNA sequencing and metabolome *via* untargeted LC-MS.

Results: Zonisamide treatment did not significantly alter microbiome composition. Both sex ($p = 0.003$) and psychotropic medication usage ($p = 0.025$) are significantly associated with baseline microbiome. Genus-level relative abundance at baseline was significantly correlated to drinking behavior and changes in drinking-associated biomarkers over the course of treatment. Baseline relative abundance of seven genera was significantly correlated with percent drinking reduction independent of treatment ($|\rho|>0.3$, $p < 0.05$). Baseline microbiome composition was significantly different between high and low responders (67-100% and 0-33% drinking reduction, respectively) independent of treatment and sex ($p = 0.03$). Further, predicted function from 16S analysis suggests a strong positive relationship between GABA degradation and percent drinking reduction ($p < 0.05$). The predicted GABA pathway association is supported by significant positive correlation of baseline stool GABA and 2-oxoglutarate with reduced drinking behavior ($p =0.03$).

Conclusions: These findings suggest microbiome composition exerts an influence on drinking behavior and intervention success. Further work in this area will better illuminate the microbiota and metabolic pathways driving these responses and enable microbiome-related personalized interventions and improved intervention outcomes in adults with AUD.

7. Jessica Flori

Defining Sober Harms

Jessica Flori[1], Michael E. Dunn[2], Kristyn Zajac[1]

[1]Calhoun Cardiology Center Behavioral, Behavioral Health, UConn Health, [2]Psychology Department, University of Central Florida, Orlando, FL

Reductions in alcohol use have often led to smaller reductions in harms than expected, and this may result from inadequate measurement of alcohol-related consequences. Harms questionnaires typically include consequences that can occur in the absence of alcohol use leading to an over attribution of certain harms to drinking (e.g., an individual might endorse engaging in risky behavior while drinking and while sober). The purpose of this study was to examine limitations of harms assessment by collecting data on frequency of harms while drinking and while sober, and to identify potential determinants of "sober" harms unrelated to alcohol use. College student participants (N = 768) completed measures of alcohol-related harms, sober harms, and potential predictor variables. Mixed effects negative binomial regression was used to compare frequency of sober and alcohol-related harms. Results revealed seven out of eight harms occurred more often while sober. When controlling for drinking frequency, six out of eight harms occurred more often while sober, while other harms occurred at comparable rates. Results indicate most of the harms measured in this study occur more frequently in the absence of alcohol use. These findings may explain the persistence of high rates of consequences reported in relation to alcohol use despite significant decreases in drinking following intervention. Future research should integrate risk and protective factors that predict all harms to better inform prevention/intervention strategies.

8. Elise Bragard

Post-college friendship-related stress and drinking to cope among emerging adults

Elise Bragard[1], Stephen Armeli[2], Howard Tennen,[1,3]

[1]Alcohol Research Center, University of Connecticut School of Medicine, [2]Department of Psychology, Fairleigh Dickinson University, Teaneck, NJ, [3]Department of Public Health Sciences, University of Connecticut School of Medicine

Background: Coping-related drinking is a strong predictor of drinking-related problems and interpersonal stress is a key correlate of drinking-to-cope (DTC) among emerging adults. However, limited research has focused specifically on interpersonal stress resulting from friendships. Young women tend to have more stable and supportive friendships than men but are more reactive to interpersonal stress and more likely to drink to cope with stress. The current study examined the unique influences of episodic and chronic stress associated with friendships and social life in predicting changes in DTC from college to post-college life.

Methods: Moderate to heavy college student drinkers reported their drinking motives and alcohol consumption each day for 30 days using an Internet-based diary in college and again five years later (N=897, 54.3% women, M_{age}=24.6). At the post-college wave, participants completed the UCLA-LSI, a well-validated semi-structured phone-based interview to assess chronic and episodic friendship and social life stress.

Results: Chronic friendship and social life stress was positively correlated with mean daily levels of post-college DTC. We found evidence that chronic stress moderated the effect of friendship conflict stress, with individuals reporting less chronic stress displaying a stronger positive association between friendship

conflict stress and post-college DTC. Conversely, chronic friendship and social life stress was negatively related to post-college heavy drinking and social drinking.

Conclusions: The effect of stressful friendship events on emerging adults' coping-related drinking may depend on their chronic friendship-related stress levels. Although friendship stress is a risk factor for drinking to cope, emerging adults with richer social lives engage in more heavy drinking in social settings.

9. Chengliang Wang

Structural basis for GSDMB pore formation and its targeting by IpaH7.8

Chengliang Wang[1], Sonia Shivcharan[1, 2], Tian Tian[1, 2], Skylar Wright[1, 2], Vijay A. Rathinam[1], and Jianbin Ruan[1]

[1]Department of Immunology, School of Medicine, University of Connecticut Health Center
[2]These authors contributed equally

Gasdermins (GSDMs) are pore-forming proteins that play critical roles in host defense by executing pyroptosis[1,2]. Among GSDMs, GSDMB is unique owing to its distinct lipid-binding profile and the lack of consensus on its pyroptotic potential[3-7]. Recently, GSDMB is shown to exhibit direct bactericidal activity through its pore-forming activity[4]. *Shigella*, an intracellular human-adapted enteropathogen, evades this GSDMB-mediated host defense by secreting IpaH7.8, a virulence effector that triggers ubiquitination-dependent proteasomal degradation of GSDMB[4]. Here, we report the cryo-electron microscopy (cryo-EM) structures of human GSDMB in complex with *Shigella* IpaH7.8 and the GSDMB pore. The structure of GSDMB-IpaH7.8 complex identifies a motif of three negatively charged residues in GSDMB as the structural determinant recognized by IpaH7.8. Human, but not mouse, GSDMD contains this conserved motif, explaining the species specificity of IpaH7.8. The GSDMB pore structure reveals the alternative splicing-regulated interdomain linker in GSDMB as a regulator for GSDMB pore formation. GSDMB isoforms with a canonical interdomain linker exhibit normal pyroptotic activity, whereas other isoforms exhibit attenuated or no pyroptotic activity. Overall, this work sheds light on the molecular mechanisms of *Shigella* IpaH7.8 recognition and targeting of GSDMs and reveals a structural determinant in GSDMB critical for its pyroptotic activity.

10. Sona Shivcharan

Human epithelial cell sensing of intracellular bacterial invasion.

Sonia Shivcharan, Skylar Wright and Vijay Rathinam

Department of Immunology, UConn Health

Epithelial cells at the mucosal sites are part of the first line of defense against microbial invasions. Epithelial cells have specialized mechanisms to sense intracellular invasion of pathogens. A non-canonical form of inflammasome with caspase-4 senses cytosolic invasion by gram-negative bacteria such as *Salmonella Typhimurium, Shigella Flexneri* and *Burkholderia Thailandensis* in human epithelial cells by monitoring the cytosol for lipopolysaccharide, a major cell wall component of gram negative bacterial pathogens. Caspase-4 sensing of bacterial LPS triggers its protease activity, which in turn leads to the proteolytic activation of a membrane pore-forming protein called gasdermin D (GSDMD). Cleaved GSDMD migrates to the plasma membrane and forms pores, leading to a lytic form of cell death called pyroptosis. Recent studies have reported that in human cells interferon-gamma inducible guanylate binding proteins facilitate caspase-4 recruitment to the cytosolic bacteria and bring about GSDMD-mediated pyroptosis and IL-18 release. A key event concurrent with bacterial escape is phagosomal rupture/destabilization which results in exposing otherwise hidden host endosomal glycans. Recognition of these glycans intracellularly brings about

accumulation of galectins 3, 8 and 9 on the damaged membrane. In this study, we are examining the role of galectins and guanylate binding proteins in the sensing of bacterial invasions by the caspase-4-noncanonical inflammasome. We will discuss our findings on how these different cytosolic proteins co-ordinate cell-intrinsic host defense against intracellular bacterial pathogens and the consequent immune responses.

11. Puja Kumari

Unveiling the immune surveillance role of extracellular vesicles

Puja Kumari[1], Swathy O. Vasudevan[1], Ashley J. Russo[1], Skylar S. Wright[1], Víctor Fraile-Ágreda[1,2], Dylan Krajewski[1], Evan R. Jellison[1], Ignacio Rubio[2], Michael Bauer[2], Atsushi Shimoyama[3], Koichi Fukase[3], Yuanpeng Zhang[4], Joel Pachter[1], Sivapriya Kailasan Vanaja[1], and Vijay A. Rathinam[1]

[1]Department of Immunology, UConn Health, [2]Department for Anesthesiology & Intensive Care Medicine, Jena University Hospital, Jena, Germany, [3]Department of Chemistry, Graduate School of Science, Osaka University, Japan, [4]FormuMax Scientific, Inc., Sunnyvale, CA

Intracellular immune surveillance for systemic microbial components during homeostasis and infections governs host physiology and immunity. However, a long-standing question is how circulating microbial ligands become accessible to intracellular receptors. Here, we show a role for host-derived extracellular vesicles (EVs) in this process; human and murine plasma- and cell culture-derived EVs have an intrinsic capacity to bind bacterial lipopolysaccharide (LPS). Remarkably, circulating host EVs capture blood-borne LPS in vivo, and the LPS-laden EVs confer cytosolic access for LPS, triggering noncanonical inflammasome activation of GSDMD and pyroptosis. Mechanistically, the interaction between EV lipid bilayer and LPS' lipid A underlies EV capture of LPS. Furthermore, the intracellular transfer of LPS by EVs is mediated by CD14-dependent receptor-mediated endocytosis. Overall, this study demonstrates that EVs capture and escort systemic LPS to the cytosol licensing inflammasome responses, uncovering EVs as a previously unrecognized link between systemic pathogen-associated molecular pattern (PAMPs) and intracellular immune surveillance.

12. Ayano Hatori

Inhibition of AMPK restores dysfunctional osteoclasts but only partially rescues skeletal phenotype in a mouse model for craniometaphyseal dysplasia

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Craniometaphyseal dysplasia (CMD) is a rare genetic disorder characterized by progressive thickening of craniofacial bones and flared metaphyses of long bones. CMD patients frequently develop hearing or vision impairment, facial palsy, and headache due to narrowed cranial foramina. Management of CMD is limited to decompression surgery. Ank knockin (KI) ($Ank^{KI/KI}$) mice that carry the CMD mutation $ANK_{F377del}$ replicate human CMD. ANK/ANKH is a transporter for small molecules like ATP and citrate. We have shown that small-sized $Ank^{KI/KI}$ osteoclasts (OCs) resorb less bone and activation of the master switch for energy metabolism AMPK (AMP-activated protein kinase) is significantly increased compared to $Ank^{+/+}$ OCs. Here, we examine effects of AMPK inhibition on the mouse CMD-like phenotype *in vitro* and *in vivo*. AMPK inhibitor SBI-0206965 (SBI) significantly increases numbers, size, and bone resorption of $Ank^{KI/KI}$ OC cultures. We administered SBI (10 mg/g) or vehicle via IP injection weekly to $Ank^{+/+}$ and $Ank^{KI/KI}$ mice from age 1 to 8 weeks. We examined skulls, mandibles, and femurs by radiographs and micro-computed tomography (mCT). $Ank^{KI/KI}$ mice that received SBI or vehicle gained less weight than vehicle-treated $Ank^{+/+}$ littermates. SBI injection enlarges the

foramen magnum and improves the positioning of cervical loops of incisors in *Ank*$^{KI/KI}$ mice. The SBI treatment, however, does not correct the hyperostotic mandibles, trabecular and cortical parameters of femurs in *Ank*$^{KI/KI}$ mice. Although inhibition of AMPK increases bone resorption via enhancement of OC function, it also increases bone formation by promoting osteoblast differentiation, which may explain the limited systemic effects of SBI administration in *Ank*$^{KI/KI}$ mice. Nonetheless, this proof-of-principle study suggests an important involvement of AMPK in the pathogenesis of CMD. An ongoing project to delete AMPK in an OC-specific manner by crossing *Ank*$^{KI/+}$ and LysMCre-*AMPK*$^{null/null}$ mice will further dissect the influence of improved OC function in CMD.

13. Francis O'Neill

Identification of novel immunogenic protein variants in osteosarcoma tumors through long-read RNA sequencing

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Osteosarcoma (OS) remains a debilitating disease within the pediatric population, with few treatment developments over the past four decades, and very limited options for metastatic disease. In anticipation of the need to develop effective immunotherapy strategies for OS, we have been engaged in the understanding of the tumor immune microenvironment and the identification of novel neoantigens of OS. For the latter, we have developed a process to identify and select for OS tumor-specific protein variants which have novel peptide sequences that are highly immunogenic. Our custom proteogenomic workflow integrates a wide variety of publicly available and widely published transcriptomic, proteomic, and genomic analysis tools. Both Pacific Biosciences (Pacbio) long-read RNA sequencing and Illumina short-read RNA sequencing were applied to three OS clinical samples to generate a high-quality OS transcriptome containing over 10K novel full length coding transcripts per tumor sample. Percent Spliced In (PSI) values were calculated for each sequenced full-length transcript with alternatively spliced variants and differential transcript usage analysis was performed using 86 OS tumor samples from the TARGET (Therapeutically Applicable Research to Generate Effective Therapies) study and 126 GTEx healthy tissue samples. Translation of the novel Pacbio coding transcripts to protein was validated by identifying peptide spectra matches (PSM) between the predicted Pacbio protein sequence and publicly available mass spectrometry spectra files which included OS cell line (U2OS) and other cancer cell lines. We identified over 30 novel alternatively spliced RNA transcripts that are differentially enriched in OS, supported by PSM, and contain a novel peptide sequence as compared to all Uniprot protein variants. Lastly, 27 candidate protein isoforms were predicted to contain immunogenic peptide sequences in regions of novel amino acid sequences. Therefore, our proteogenomic workflow could identify novel protein variants containing immunogenic peptide sequences that can be considered as promising targets for novel immuno-oncology therapies.

14. Suranji Wijekoon

Engineering composite nanofibrous scaffolds with decellularized extracellular matrix for critical size femoral defect repair in rat models

Suranji Wijekoon, Sama Abdulmalik, Rosalie Bordett, Aditya Ruikar, Sara Katebifar, Sangamesh G Kumbar

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Approximately 20% of combat personnel experience fractures and bone loss in their extremities, resulting in the Department of Defense spending approximately $116 million in every year. These complex bone injuries

can also be created by infection, tumor resection, atrophic non-unions, and osteoporosis. Therefor there is a clinical and financial need for innovative bone regeneration therapies with a biomimetic scaffold containing vascular and mineral nanomaterials for enhanced neovascularization and osseointegration in large bone defects. In this study, a critical size femoral defect model in rats will be used to implant the nanofibrous-decellularized extracellular matrix (dECM) scaffolds at the defect site. Cell derived dECM will be prepared using primary rat osteoblasts, articular chondrocytes, endothelial cells, fibroblasts, and bone marrow stem cells (BMSCs) to create both hard-shell and soft-shell scaffolds. At 12 weeks after surgery, animals from each group will be sacrificed and bone regeneration will be assessed. Histology and histomorphometry will be used identify the cell infiltration and deposition of osteogenic markers within the scaffolds, indicate to successful tissue integration. Micro-CT scans will be used to demonstrate the bone formation and amount of mineralization in the scaffold-treated groups compared to controls. Moreover, biomechanical testing will be showed changes of mechanical properties in the treated femurs, highlighting the strength and stability imparted by the nanofibrous-dECM scaffolds. We expect the scaffolds of the hard-shell scaffold with the highest CaP deposition and the soft-shell scaffold with optimal dECM to achieve complete regeneration of the defect characterized by aligned and mineralized cortical bone, a lack of presence of cancellous bone in the cortical region, and numerous haversian canals. Therefore, we anticipate that this group will perform equivalently or even superiorly to autografts/allografts for repairing segmental bone defects.

15. Chinedu Ude

Anterior Cruciate Ligament Reconstruction with Tiger Graft: a Semi-Biodegradable Scaffold
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Purpose: Competitive sportsmen, energetic youths, and military parachuters frequently suffer injuries to the anterior cruciate ligament (ACL), a crucial knee joint support mechanism. The FDA has not yet approved a totally synthetic graft for ACL restoration due to graft rupture problems with earlier biodegradable grafts. Here, we elaborate on the design of a composite synthetic graft that provides additional structural support and is made of both biodegradable and non-biodegradable polymers.

Method: Biodegradable poly-L-lactic acid (PLLA) was combined with non-biodegradable polyethylene terephthalate (PET) to form a ‘tiger graft’. Tiger graft was implanted in the left knee of 12 New Zealand white rabbits for an extended in vivo evaluation of 6 months with ensuing biomechanics and histomorphometry analysis.

Result: At 6 months, the tiger grafts' volume had decreased in comparison to the initial graft, but a gross examination showed that 11 out of 12 were still intact. Micro-CT scans showed that there was evidence of bone tunnel osteogenesis. Biomechanics testing revealed diminished strength compared to the prior data at 3 -month in vivo time point, despite the fact that histology and SEM analysis had shown signs of tissue regeneration and a potential replacement for the biodegradable component PLLA. In particular, the Goldner Trichrome staining showed that fresh tissues had been infused into the graft joint space.

Conclusion: Tiger graft offers additional early structural support though further optimization is required to match biomechanics strength and enhance ligament regeneration at later time points.

16. Aaron Taylor

Immunosuppressive tumor microenvironment of osteosarcoma

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Introduction - Osteosarcoma (OS) is the most common malignant bone tumor in children. OS is characterized by a high degree of genomic instability, resulting in copy-number alterations and genomic rearrangements with no disease-defining recurrent mutations. Given the diverse genomic landscape of OS and the difficulty of identifying druggable therapeutic targets, use of immunotherapy techniques appears lucrative. However, clinical trials based on molecular characterization have failed to find new effective therapies, and outcomes have not improved over the last 40 years.

Materials/Methods – We performed single-cell RNA sequencing (scRNA-seq) using the 10x Genomics Chromium platform on six fresh tumor biopsy samples from pediatric OS patients. Raw data was processed using 10x CellRanger to produce transcript read counts for each cell. After filtering low-quality cells and doublet removal, counts were normalized using *Seurat*, and cells were integrated across samples with *Harmony*. Data was combined with a previously-published OS scRNA-seq cohort of six samples (GSE162454). Two additional OS samples were profiled using 10x Genomics Visium spatial transcriptomics for validation of discovered subtypes and to add spatial context.

Results - Clustering identified 16 major cell types based on expression of canonical cell markers. Several immunosuppressive cell types were identified via subclustering of major cell types, including neutrophil myeloid-derived suppressor cells (MDSCs), regulatory and exhausted T-cells, and LAMP3+ dendritic cells. Markers for the cell types found in OS were identified for further validation using imaging techniques, including Visium spatial transcriptomics. We performed deconvolution using the scRNA-seq cell identities to examine colocalization of discovered cell types. Overall, the discovered clusters were common between patients, showing consistent cell type proportions. However, we found patient-specific differences in the frequency of some cell types, with one sample showing a higher proportion of T-cells along with increased presence of colocalized IFN-stimulated macrophages, and the other with a greater presence of neutrophils/MDSCs.

17. Sama Abdulmalik

Electrical and Chemical Stimulation Using Ionically Conductive Polymeric Implants for Soft Tissue and Wound Healing

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Musculoskeletal injuries are rising in the US, impact quality of life, and result in pain, loss of function, and significant financial burden. Repairing musculoskeletal injuries often involves surgical interventions, which lead to scarring, a natural process of wound healing. Scar tissue is a highly disorganized fibrotic extracellular matrix (ECM) with altered components that present a physical barrier for regeneration. Mitigating scarring and promoting regeneration via pharmacological agents, biomaterials, cells, and other physical forces have varying degrees of success and several difficulties in their implementation. *Thus, we developed a novel ionically conducting (IC) chitosan scaffold to enable the delivery of both electrical stimulation (ES) and*

pharmacological agents to promote regenerative healing with reduced scar formation. The benefits of ES in repairing and regenerating are well-established in animals and humans; However, it poses some challenging such as infection risk of penetrating metallic electrodes and associated patient non-compliance, off-target nonspecific effects, and expensive bulky ES units. *Thus, we engineered an IC implant to deliver ES and small molecule 4-aminopyridine (4AP) to promote regenerative healing with reduced scarring.* Herein, Wistar rats were used for full-thickness skin wound defect healing. 2 cm^2 full-thickness skin wounds were made in rats and implant materials were used on the wound. Wound contraction rate was measured over 14 days. Two weeks post-surgery, scaffolds were harvested. Histological evaluation was used to assess blood vessel infiltration and wound healing. We confirmed that our implants sustain ionic conductivity (130-145 mS/cm for 12 wks), and are porous to aid cell infiltration (average diameter = 59.3 ± 14.2 μm [mean ± SD]). These implants also sustained release of highly water soluble 4-AP over 8 weeks. In a rat full-thickness wound healing model, ES+4-AP implants resulted in significantly faster wound closure rates, and had higher Col-I/Col-III. Hence, it promotes regeneration and reduce scar formation. Ongoing studies will focus on establishing the mechanisms and benefits of engineered IC implants and electrodes with ES and other small drug molecules in tissue regeneration.

18. Manaswee Barua

Polyphosphazene-PLGA blends for bone regeneration

Manaswee Barua[1] and Cato T. Laurencin[1,2,3,4,5,6,7]

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Bone is a dynamic tissue which continuously undergoes resorption and deposition. During a fracture, bones regenerate via a series of complex well-orchestrated physiological processes. However, if the size of the bone defect is above a certain limit (critical size, length ~3 times diameter), the regeneration process needs to be augmented surgically and one emerging approach towards it is using polymeric bone graft substitutes. Polyphosphazenes are an interesting class of inorganic-organic hybrid biodegradable polymers with immense biomedical applications. Polyphosphazene structure, synthetic flexibility, amphoteric degradation products, low *in vitro* and *in vivo* cytotoxicity, tunable physical and chemical properties make them interesting candidates for bone regeneration applications. In this project, we prepare polyphosphazene-PLGA blend based scaffolds which undergo *in situ* pore formation upon degradation. These blend scaffolds show osteoconductivity *in vitro* and we are currently studying the efficacy of these scaffolds for the regeneration of large sized bone defects (4cm*3cm) in rabbit tibia model.

19. Maeva Devoucoux

Defining the mechanistic links between the epigenome and alternative splicing during breast aging and tumorigenesis.

Maeva Devoucoux[1], Brittany Angarola[1], Zenghsuo Mo[1], Sunghee Park[1], Rachel Gott[3], Mattia Brugiolo[1], Marina Yurieva[1], Mark A. LaBarge[2], Ron Korstanje[3], Olga Anczukow[1]

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Aging is a progressive biological process resulting in the gradual loss of tissue function. At the molecular level, aged cells display changes in chromatin marks, gene expression, and post-transcriptional regulation, including in RNA alternative splicing. How these changes contribute to age-related pathologies, including neurodegenerative diseases and cancers, remains poorly understood. Although age is the main risk factor for breast cancer, how it impacts chromatin structure and RNA splicing in mammary tissues remains understudied. Interestingly, chromatin has been shown to impact splicing via two models: the kinetic model, linked to the RNA polymerase II elongation rate, and the chromatin-adaptor recruiting model, leading to modulate splicing-factor recruitment. Yet, how these two molecular processes interact with each other during aging is still poorly understood. I will employ cutting-edge technologies such as Cleavage Under Targets & Tagmentation (CUT&Tag), a chromatin-profiling method, and long-read RNA-sequencing to detect RNA isoforms. To uncover the mechanistic links between chromatin structure and alternative splicing regulation during normal breast aging, I will integrate these chromatin-profiling data with alternative splicing data from aged mammary cells, in healthy mice and women. These results will yield a better overview of the epigenetic and post-transcriptional landscapes during normal breast aging. These findings will advance our understanding of how those alterations can drive breast-cancer initiation.

20. Kevin Child

Understanding splicing regulation during human heart organogenesis
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Congenital Heart Defects (CHDs) are the most common among congenital abnormalities worldwide affecting approximately 1% of all births. Current research addressing this problem has focused on heart samples from fetal and adult stages with very little focus on the earliest stages of organogenesis. Recent work has elucidated the importance of gene expression dynamics during the first eight weeks of heart development. In addition, work has revealed general splicing patterns across multiple species and organs which found a unique splicing program for both heart and brain development that appears to be conserved across several species and which differs from all other tissues investigated. Our lab has been focused on the organogenesis period of heart development between Carnegie stages (CS) 13 and 23. We found a module of genes with dynamic expression through this period that is identified as RNA splicing and processing. Looking at alternative splicing (AS) between our 8 CS used in the previous study we find thousands of events with an increase in AS over the various stages of heart development. We also found evidence for splicing events which result in transcripts which have not been previously annotated. This result led to the generation of a novel annotation using a combination of long and short read data with these same CS samples. From this analysis we have identified thousands of novel transcripts with a majority of them being transcript variants of well-known genes. One example of this is TBX5 a gene where several mutations have already been identified which led to Holt-Oram Syndrome. We discovered a novel TSS for TBX5 which is highly conserved and constrained. The discovery of these novel exons and understanding of temporal splicing patterns could help us in screening for several of the undiagnosed CHDs.

21. Amit Gupta

NaP-TRAP-Seq: An approach to study the role of mRNA *cis*-elements in translational regulation
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Accurate control of translation is crucial for maintaining normal cellular function and fate. The regulation of translation is primarily carried out by a combination of *trans*-acting factors and *cis*-regulatory elements. While various methods are available to investigate the role of *trans*-acting factors (e.g., RNA binding proteins) in translation, understanding the function of *cis*-regulatory elements in mRNA translation is particularly challenging due to the lack of effective techniques for measuring their activity in a high-throughput manner. To enhance our understanding of the *cis*-regulatory code present in untranslated regions (UTRs) and its role in translational regulation, we have developed a novel approach called Nascent Peptide-mediated Translating Ribosome Affinity Purification (NaP-TRAP). This approach is highly effective in identifying and measuring the regulatory activity of UTR elements on translation. We have validated the robustness of NaP-TRAP in multiple model systems, including zebrafish embryos, HEK293 cells, and human stem cells. Next, we adapted NaP-TRAP into a new type of massively parallel reporter assay (NaP-TRAP-seq) to investigate the regulatory activity of over 10,000 5'-UTR sequences in early vertebrate embryos. As expected, we found that uORF have a strong repressive effect on translation, validating our approach. Moreover, we identified an antagonistic effect for U-rich and C-rich regions where stretches of Us activate and C-rich sequences repress translation in the early embryo. Currently, our research focuses on understanding the specific role of translation initiation factors in the process of translation initiation using NaP-TRAP-seq. To achieve this, we plan to combine NaP-TRAP-seq experiments with selective inhibition of translation initiation factors, eIF4A and eIF4E, to identify their targeted 5'-UTR features they recognized. Ultimately, our goal is to understand how 5'-UTR elements control translation regulation in various systems.

22. Cesar Zoni

Relationship between donor ejection fraction, left ventricular wall thickness and mortality in heart transplants recipients

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# Both authors equal contribution as senior authors

Background: Limited availability of donor hearts contributes to significant mortality in patients waitlisted for heart transplantation (HTx). Donor hearts with reduced ejection fraction (EF) and left ventricular hypertrophy are considered to adversely affect long-term HTx outcomes and are often rejected.

Objective: To determine the relationship of donor's EF and left ventricular wall thickness (LVWT) with mortality in HTx recipients.

Method: This was a retrospective analysis from The United Network for Organ Sharing (UNOS) registry. Adult HTx recipients with donor's EF and LVWT recorded from 2006 to 2022 were included. Study sample was grouped according to donor characteristics (group 1 EF>50% & LVWT<1.4cm, group 2 EF≤50% & LVWT<1.4cm, group 3 EF>50% & LVWT≥1.4cm, and group 4 EF≤50% & LVWT≥1.4cm). The primary end point was mortality (30 days, 1 year, and 5 years).

Results: 21,012 patients were included, in which 19,205(91.4%) were in group 1, 898(4.3%) in group 2, 867(4.1%) in group 3, and 42(0.2%) in group 4. There were significant differences in baseline characteristics among the groups. Unadjusted mortality was 6.3%, 6.0%, 6.0%, and 2.4% (p=0.86) at 30 days; 16.2%, 13.5%, 16.8%, and 7.3% (p=0.08) at 1 year; and 32.2%, 29.2%, 35.4%, and 29.0% (p=0.18) at 5 years, respectively. In addition, adjusted mortality did not differ across the groups.

Conclusion: There were no significant differences in recipient mortality in groups based on donor EF and LVWT. Expanding the donor selection criteria would allow for increase in the donor pool and assist in decreasing the mortality, while on the waitlist for HTx.

23. Ashok Cheemala

Reduced endothelial levels of splice factor TDP-43 associated with ALS/FTD- mutation TARDBP^{G348C} causes hallmarks of FTD in an animal model

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TDP-43 is aggregated in neurodegenerative neurons, limiting critical functions in RNA splicing, and specific mutations in the gene are linked to familial Frontal Temporal Lobar Dementia (FTD), and Amyotrophic Lateral Sclerosis (ALS). Although the focus has been on neurons, the gene is expressed in brain endothelial cells (ECs) EC dysfunction of TDP-43 may be important, as increased permeability across the blood brain barrier (BBB) predicts cognitive decline. To test this, we examined EC function and BBB properties in mice with either 1) ALS/FTD knock-in in TDP-43 (TDP-43G348C/+) or 2) EC-specific deletion of endothelial TDP-43 throughout the endothelium (Cdh5(PAC)CreERT2; Tardbpff) or specifically within the brain endothelium (Slco1c1(BAC)CreERT2; Tardbpff). We found increased permeability to 3kDa Texas Red dextran in TDP-43G348C/+ mice. ECs derived from these mice exhibited reduced nuclear TDP-43, impaired expansion, reduced cell junction proteins, and impaired barrier function in vitro. Adult EC deletion in Cdh5(PAC)-CreERT2; Tardbp ff mice resulted in systemic EC activation, permeability, fibrosis, and lethality within 3 weeks. To focus more specifically on the contributions of the brain ECs, we examined Slco1c1(BAC)-CreERT2; Tardbp ff mice. Unlike systemic EC deletion, this was not lethal, but mice exhibited increased BBB permeability within just a week of Tam-mediated excision and chronic increases in vascular fibrin, vascular rarefication and reactive microglia and astrocytes, and ultimately cognitive dysfunction. Together, these data show that TDP-43 loss within brain ECs could contribute to the BBB defects observed in the progression of ALS/FTD.

24. Sungryong Oh

Uncovering human biased gene regulatory features in human heart development by integrating primary tissues and organoids model

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Organogenesis is a highly conserved process across mammals, but ultimately results in distinct differences in tissue phenotypes across species. However, the molecular drivers of this specificity are largely unknown. Changes in gene regulation have been suggested to be major contributors to species-specific differences but systematic identification of such regulatory changes have been limited. Recent studies have reported that mutations on non-coding gene regulatory regions, called enhancers, are one of the main contributors of non-syndromic congenital disorder. Tissue-specific enhancers have lots of variance in conservation across vertebrates, suggesting there could be substantial human-specific features in organ development. My study seeks to address this issue by identifying human biased gene regulatory landscapes and the factors that control their activation in the context of organ development. My project focuses on fully uncovering of heart

development. Heart is known to have similar structures but have different functions and molecular features between human and mice heart. In addition, heart is also known to have less conserved enhancers than some other tissues. These findings lead into the idea that developing heart has human biased unique gene expression regulatory features, and variation of these regions contributes on non-syndromic Congenital Heart Disorder (CHD) features. To investigate these human unique features in entire heart organogenesis, my project combines genome-wide studies on primary tissues and *in vitro* organoid model. With the following results, it is expected understand entire human heart development which cannot be modelled in rodent systems. Moreover, my project is also expected to reveal enhancers that potentially play a critical role and have a correlation to CHD.

25. Aditya Kanwal

Developing a CRISPR activation therapeutic for dilated cardiomyopathy.

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Titin truncation variants (TTNtvs) are the most common genetic lesion identified in individuals with dilated cardiomyopathy (DCM). They are associated with high morbidity and mortality in patients. Expression of TTNtvs is accompanied with the reduction in normal TTN protein levels, and impaired sarcomere content and function. Currently no therapeutics exist targeting TTNtvs owing to the immense size of TTN, the variation of mutations leading to TTNtvs, and incomplete knowledge of TTNtv pathogenicity. To interrogate this, we have adapted CRISPR activation using dCas9-VPR and develop a therapeutic in human cardiomyocytes (CMs) and 3-dimensional cardiac microtissues (CMTs) engineered from induced pluripotent stem cell models harboring a DCM-associated TTNtv. Our data reveals that TTN CRISPR activation can rescue TTNtv-related functional deficits despite increasing truncated TTN levels, which provides evidence to support haploinsufficiency as the predominant genetic mechanism of heterozygous TTNtvs. CRISPR activation could be developed as a therapeutic to treat a large proportion of TTNtvs.

26. Moriah Turcotte

Perinuclear β-adrenergic receptors are necessary and sufficient to promote cardiac hypertrophy

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Pathological cardiac hypertrophy development is controlled by networks of signaling pathways, integrated by scaffold proteins that localize signaling enzymes and facilitate cross-talk between these pathways, leading to upregulation of hypertrophic transcription factors. Over the last several decades, our lab has studied a protein scaffold at the nuclear envelope, muscle A-Kinase Anchoring Protein (mAKAPβ), required for the induction of cardiac hypertrophy. Among the various pathways affected, mAKAPβ particularly tethers downstream targets of β-adrenergic receptors (βAR), and this localization provides the framework for stress-related gene transcription in cardiomyocytes. Although the traditional dogma of adrenergic receptor signaling posits receptor activation begins at the plasma membrane, recent evidence has emerged showing intracellular

adrenergic receptor localization and activation. We now provide evidence of a perinuclear cAMP domain, dependent on mAKAP expression. Utilizing novel peptides localized to mAKAP, we stimulate or inhibit perinuclear βARs within proximity. We demonstrate perinuclear βARs are necessary and sufficient for cardiac hypertrophy development in neonatal and adult rat cardiac myocytes, constituting a functionally independent cAMP domain. Additionally, we are investigating possible subcellular locations of βARs that could be supporting cAMP concentrations at mAKAPβ. This research has high therapeutic value as our peptides prevent disease without impacting canonical cardiomyocyte function.

27. Elham Ahmadi

Virus like vesicle based trivalent agent for cancer immunotherapy
Elham Ahmadi and Kepeng Wang

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Immune checkpoint blockade (ICB) and oncolytic viral therapies are only effective in limited numbers of cancer patients. Targeting multiple immune mechanisms may overcome therapy resistance and further improve cancer immunotherapy for humans. Here we describe the use of virus-like vesicles (VLV)—a novel hybrid alphavirus-rhabdovirus vector—for the delivery of three immunomodulators in cancer therapy. This high-capacity oncolytic RNA replicon vector was adapted to simultaneously delivering interleukin (IL)-12, short-hairpin RNA (shRNA) targeting programmed death ligand 1 (PD-L1), and a dominant-negative form of IL-17 receptor A (dn-IL17RA) from same vector. This trivalent viral vector (designated CARG-2020) was injected intralesionally to subcutaneous MC38 and BNL-T3, and it eradicated large established tumors in mice. The approach of combining transgenes results in a therapeutic agent that prevents tumor recurrence and thus prolonged overall survival. Noninjected distant tumor inhibition studies after primary tumor treatment as well as tumor re-challenge studies demonstrate the establishment of a strong immunological memory from the agent. Mechanistically, CARG-2020 potently activates Th1-armed immunity, and inhibits the expression of genes related to T cell exhaustion and cancer-promoting inflammation. The ability of CARG-2020 to prevent tumor recurrence in mice and to extend their survival makes it a promising candidate for use in human cancer immunotherapy.

28. Samaneh Poursaeid

Deciphering the Transcriptional Network Governing Cell Fate Boundaries in *Drosophila* Germline Stem Cell Niche
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Drosophila male germ stem cells (GSCs) constantly undergo asymmetric divisions, generating daughter cells with distinct cell fates. The mechanisms that retain one daughter cell as a stem cell while promoting differentiation in the other remain unknown. The *Drosophila* testis has been an iconic model system for studying the mechanisms in which diverse cell types collaborate to maintain the integrity and function of the gonad. Our current study has revealed the essential role of Decapentapelagic (Dpp), a member of Bone Morphogenic Protein (BMP) ligand family, not only in the maintenance but also in the differentiation of GSCs in *Drosophila*. Dpp signaling regulates the asymmetric division of GSCs by targeting the transcription of the differentiation factor known as bag-of-marbles (bam). The bam silencer element (SE) represents a robust binding site for Mad (Mothers against Dpp), a downstream signaling effector. Our previous analyses have strongly indicated that a functional switch in Mad's role, from a suppressor to an activator of bam transcription as GSCs differentiate into the spermatogonia (SGs). To understand how Mad switches its role on the same

target gene, we hypothesize the potential involvement of a co-factor in this process. Subsequently, we conducted an in-silico analysis of the bam promoter region, which revealed that the SE in the bam promotor may also present a high affinity for a well-known transcriptional repressor, Brinker (Brk). Interestingly, we found that brk is expressed in early SGs where Bam is expressed. RNAi-mediated knockdown of brk resulted in high levels of bam expressions in SGs. The epistasis analyses demonstrated that Dpp signaling lowers brk expression levels in SGs, thereby indirectly upregulates bam expression to promote differentiation. Taken together, our data suggest that the involvement of various transcriptional factors in regulating the temporal and spatial expression of bam, thereby intricately orchestrating germ stem cell fate.

29. Yuanyuan Wu

Kindlin-3 regulates neutrophil inflammatory adhesion signaling through beta2 integrin clustering

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Neutrophils are the most abundant leukocytes in human blood. Neutrophil malfunction may cause various inflammatory diseases. Beta2 integrins are critical for neutrophil recruitment during inflammation. Kindlin-3 and talin-1 are important regulators in beta2 integrin activation signaling. We have confirmed this using beta2 integrin activation-specific antibodies KIM127 and mAb24. Besides activation, clustering is also critical for beta2 integrin avidity and inflammatory adhesion of neutrophils. Whether kindlin-3 and talin-1 are involved in beta2 integrin clustering remains unclear. Here we used super-resolution stochastic optical reconstruction microscopy (STORM) to study their roles in beta2 integrin clustering on human neutrophil-like HL60 cells. We find that lymphocyte function-associated antigen 1 (LFA-1) and macrophage-1 antigen (Mac-1) are significantly clustered in wild-type cells after IL-8 or fMLP stimulation. Using CRISPR-Cas9-mediated kindlin-3 and talin-1 knockout HL60 cells, we found that kindlin-3 but not talin-1 are responsible for beta2 integrin clustering. Furthermore, we demonstrated that Pleckstrin homology (PH) domain in kindlin-3 is critical for beta2 integrin clustering. Our study provides new insights into the regulation of beta2 integrin clustering and leukocyte recruitment.

30. Alsu Ramazanova

The role of disparate erythropoietin receptors in the regulation of megakaryocytic-erythroid progenitors.

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Chronic kidney disease (CKD) affects a significant number of adults in the United States, resulting in anemia in over 5 million patients. The standard treatment for CKD-related anemia involves administering Erythropoietin (EPO), a hormone produced by the kidneys in response to low oxygen levels. EPO is known to stimulate the production of red blood cells by supporting the survival and proliferation of erythroid-committed progenitor cells in the bone marrow. However, pharmaceutical use of EPO can lead to unintended side effects, such as an increased risk of stroke due to excessive platelet production. This highlights the need for further research to understand the effects of EPO on different types of blood cell progenitors, particularly the Megakaryocytic-Erythroid bipotent progenitor and Megakaryocytic-committed progenitor. To address this research gap, we investigate the direct effects of EPO on isolated progenitor cell populations capable of giving rise to megakaryocytic and erythroid cells. We are investigating the unique activation of signaling molecules, changes in gene expression, and observable changes in cell characteristics between cell populations in

response to EPO stimulation. Additionally, we will explore the receptors and receptor-specific downstream of EPO signaling using novel receptor-specific EPO mimetics. We hypothesize that EPO stimulation will result in the expansion of Megakaryocytic-Erythroid progenitors, leading to an increased pool of progenitors capable of producing both platelets and erythrocytes, and our approach will identify novel EPO receptor-specific targets in defined bone marrow progenitor populations. In summary, our project focuses on identifying the proteins and genes responsible for the functional responses of blood cell progenitors following EPO treatment. This knowledge will improve our understanding of the mechanisms of action of Erythropoietin in human bone marrow leading to advancements in treatments for anemia.

31. Tingting Geng

An essential role of UBXN3B in B cell survival

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The immune system comprises various cell types that coordinate responses to infections and maintain tissue and immune homeostasis. B lymphocytes play a critical role in the adaptive immune system. In this study, we report a crucial role of a UBX domain-containing protein UBXN3B in the maintenance of B cell survival throughout their development, from the bone marrow to peripheral mature B cells. Tamoxifen-induced *Ubxn3b*$^{-/-}$ mice exhibit a significant reduction in B cells, but an increase in myeloid cells compared to *Ubxn3b*$^{+/+}$ littermates. Following UBXN3b deletion in mice, B cells undergo rapid cell death. Within 10 days, their numbers decrease by half, and after 30 days, only 5% of the B cell population remains. In contrast, normal B cells have a half-life of 5-6 weeks. The transfer of wild type bone marrow to *Ubxn3b*$^{-/-}$ mice corrects the B cell deficiency, whereas reverse transplantation does not. This dysregulated immune compartmentalization renders mice highly vulnerable to SARS-CoV-2, typified by reduced production of virus-specific antibodies, increased lung immunopathology and delayed resolution of disease. Our results demonstrate a new function of UBXN3B in the maintenance of hematopoietic homeostasis and in particular B cell survival and antibody response during steady state and viral infection.

32. Alexandra Pozhidaeva

Structural characterization of phosphoethanolamine methyltransferase from *P. falciparum* using solution NMR spectroscopy and MD simulations

Alexandra Pozhidaeva[1], Yulia Pustovalova[1], Irina Bezsonova[1], Jihyun Kim[2], Oksana Gorbatyuk[1], Lucio Frydman[2] and Jeffrey C. Hoch[1]

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Over 500,000 people die from malaria every year. Recent emergence of drug-resistant *Plasmodium falciparum* underscores the urgency of developing new treatments to complement the existing therapies. Phosphatidylcholine, a major phospholipid of *P. falciparum* membranes, is synthesized through a pathway in which phosphoethanolamine methyltransferase (PfPMT) converts phosphoethanolamine to phosphocholine. This pathway is absent in mammals making the enzyme an attractive therapeutic target. Crystal structures of the PfPMT single S-adenosylmethionione-dependent catalytic domain with its substrates/inhibitors have been determined. Yet, the structure of the apo protein has never been crystalized suggesting conformational dynamics. Here, we characterize apo PfPMT and gain insight into the mechanism of its inhibition by

amodiaquine using solution NMR methods in combination with molecular dynamics simulations. This work provides a basis for future efforts to develop new potent anti-malarial compounds.

33. Amin Boroomand

Multiscale modeling of marine microbiome using Vivarium: a journey from E. coli to ocean biogeochemical cycling.

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Multiscale modeling in biology is analogous to assembling a multifaceted puzzle, with challenges arising from the need to integrate diverse data across varied biological domains. Our research seeks to address this challenge by focusing on the multiscale modeling of the marine microbiome, understanding its intricate processes, and their effects on global carbon cycles. Using Vivarium (Agmon, 2022), a robust tool for integrative multiscale modeling, we have begun our investigations with the flux balance analyses of E. coli (Skalnik, 2023). Through this model, we've deciphered the interplay between gene expression, enzyme concentrations, metabolic reactions, and their culminating impact on biomass generation in glucose-rich environments. Our subsequent goal extends to comprehending the degradation, remineralization, and interactions of particulate organic matter (POM) in the ocean's water column, a central process in the marine biogeochemical cycle. As we venture into understanding the broader marine ecosystem, our methodology involves employing the multiscale modeling approach, ensuring a holistic view of the ocean microbiome system. Leveraging Vivarium's capabilities, we anticipate a seamless integration of existing models, filling gaps, and fostering collaborations, thus advancing our understanding of marine ecosystems.

34. Everton Bettin

***Escherichia coli* OmpA β-barrel is a promising scaffold for the generation of ECL-specific antibodies against orthologous 8-stranded β-barrel OMPs from *Treponema pallidum*, the syphilis spirochete**

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Recent increases in the global incidence of syphilis highlight the urgent need for a vaccine to prevent transmission of *Treponema pallidum* (*Tp*), the syphilis spirochete. An efficacious syphilis vaccine should elicit protective antibodies against the spirochete's limited repertoire of β-barrel-forming outer membrane proteins (OMPs). Highly promising *Tp*OMPs include the 8-stranded β-barrels (*Tp*8SβB) paralogs (TP0126, TP0479, TP0698 and TP0733). Production of recombinant *Tp*8SβBs in a native conformation presents numerous challenges. To circumvent these difficulties, we grafted the extracellular loops (ECLs) from *Tp*8SβBs onto the well-characterized 8SβB for *Escherichia coli,* OmpA. OmpA/*Tp*8SβB chimeras were engineered by replacing the ECLs from OmpA-βB with the predicted loops from each *Tp*8SβB. OmpA/*Tp*8SβB were over-expressed in *E. coli* as inclusion bodies and refolded by dilution in 1% LDAO. Proper refolding of recombinant chimeras was confirmed by demonstrating heat-modifiability. Antigenicity of all four OmpA/*Tp*8SβB chimeras was evaluated by immunoblot using immune rabbit sera (IRS) and human syphilitic sera (HSS) from patients in Colombia and Malawi. Sera from mice immunized with refolded chimeras were evaluated using recombinant *Pyrococcus furiosus* thioredoxin (*Pf*Trx) containing single OMP ECLs. *Tp*8SβBs models show high superposition scores when compared to the OmpA-βB, suggesting that the *Tp*8SβB ECLs are properly

displayed in the OmpA/*Tp*8SβB scaffolds. High yields of refolded recombinant OmpA/*Tp*8SβBs were achieved and showed heat-modifiability, thus confirming their proper refolding into stable β-barrels. All four OmpA/*Tp*8SβB chimeras were recognized by HSS and/or IRS. Mice immunized with OmpA/Tp8SβB developed antibodies against individual *Tp*8SβB ECLs. Our results support the use of *E. coli* OmpA-βB as a highly versatile scaffold for presenting heterologous *T. pallidum* OMP ECLs. High yields of refolded OmpA/*Tp*8SβBs chimeras were easily achieved, enabling the generation of *Tp*8SβBs ECL-specific antisera. These antibodies will be investigated for their ability to promote neutralization and opsonophagocytosis of *T. pallidum in vitro*.

35. Sathyabaarathi Ravichandran

Distinct baseline immune characteristics associated with responses to conjugated and unconjugated pneumococcal polysaccharide vaccines in older adults

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Pneumococcal infections cause serious illness and death among older adults. A capsular polysaccharide vaccine PPSV23 (Pneumovax®) and a conjugated polysaccharide vaccine PCV13 (Prevnar®) are used to prevent these infections, yet underlying responses, and baseline predictors remain unknown. We recruited and vaccinated 39 older adults (>60 years) with PPSV23 or PCV13. Both vaccines induced strong antibody responses at day 28 and similar plasmablast transcriptional signatures at day 10, however, their baseline predictors were distinct. Analyses of baseline flow cytometry and RNA-seq data (bulk and single cell) revealed a novel baseline phenotype that is specifically associated with weaker PCV13 responses, characterized by i) increased expression of cytotoxicity-associated genes and increased $CD16^+$ NK frequency; ii) increased T_h17 and decreased T_h1 cell frequency. Men were more likely to display this cytotoxic phenotype and mounted weaker responses to PCV13 than women. Baseline expression levels of a distinct gene set was predictive of PPSV23 responses. This first precision vaccinology study for pneumococcal vaccine responses of older adults uncovered novel and distinct baseline predictors that might transform vaccination strategies and initiate novel interventions.

36. Atri Ta

A bacterial autotransporter impairs innate immune responses by targeting the transcription factor TFE3

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Type I interferons (IFNs) are consequential cytokines in antibacterial host defense. Whether and how bacterial pathogens inhibit innate immune receptor-driven type I IFN expression remains mostly unknown. By screening a library of enterohemorrhagic Escherichia coli (EHEC) mutants, we have uncovered EhaF, an uncharacterized protein, as an inhibitor of innate immune responses including IFNs in vitro. Further analyses identified EhaF as an autotransporter—a bacterial secretion system with no known innate immune roles. We found that EhaF secreted by EHEC translocates into macrophage cytosol and inhibits IFN and IL-6 expression. Importantly, we identified TFE3—a transcription factor with emerging roles in immune gene expression—as the target of EhaF; EhaF interacts with and inhibits TFE3 leading to impaired IRF3 phosphorylation and IFN expression. Notably, EhaF-mediated IFN suppression plays an essential role in EHEC colonization and pathogenesis in mice. In summary, this study has uncovered a previously unknown autotransporter-based bacterial strategy that targets a specific transcription factor to subvert innate immune responses.

37. Agnieszka Lukomska

MicroRNAs associated with maturation of retinal ganglion cells regulate survival and long-distance axon regeneration after optic nerve injury

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Retinal ganglion cells (RGCs) are central nervous system (CNS) projection neurons, which do not regenerate axons severed in optic neuropathies, such as those resulting from optic nerve trauma, ischemia, and glaucoma. Several factors that are developmentally regulated in RGCs were discovered to contribute to their failure to regenerate the injured axons. No clinical treatments exist to date that could help patients with such axonal injuries. Thus, the failure of RGC and other CNS long-distance axons to regenerate after injury remains a major unmet problem. Here, we identified novel miRNA-regulators of RGC survival and axon regeneration present potential therapeutic targets for treating optic neuropathies and glaucoma, as well as axonal injuries in other white matter tracts of the CNS.

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