August 19th 2021

1st Yale Postgraduate Trainee Research Symposium

Abstract Book

Session 1 Program Abstracts

Can We Save Orange Juice? Developing Disease-Resistant Valencia Oranges Using CRISPR Gene Editing

C. Vernon, C.H.D. Sagawa, G. Thompson, A. Khadgi, Y. Jacob, V. Irish

Huanglongbing (HLB), or “Citrus Greening Disease", is a plant bacterial infection spread by insects. This disease is devastating the production of Valencia oranges and other citrus varieties worldwide. Infected trees produce only green, immature fruits and yellow leaves before eventual plant death. To address this problem, we are using CRISPR/Cas9 gene-editing to inactivate specific genes and create a library of genetically modified orange trees, which will be screened for HLB tolerance and other bacterial citrus diseases. Our library targets a total of 1200 candidate genes that are likely involved in citrus susceptibility to HLB. We are generating 300 vectors, pieces of DNA, each of which contains instructions for CRISPR to target four genes per plant as well as an antibiotic resistance gene and encoding a fluorescent reporter protein (GFP). These vectors are being transformed into Valencia orange using Agrobacterium-mediated transformation and of the 120 plant transformations completed, 25 vectors have regenerated shoots and nine of those expressed fluorescence. Transformed shoots are then micrografted onto Carrizo Citrange, the rootstock commonly used in commercial orange groves. While our hope is to obtain plants tolerant to HLB, we will also screen for tolerance to other destructive citrus diseases and evaluate plants that show interesting phenotypes. This citrus plant library will be available as a resource for other citrus researchers. All the genetic information of these modified plants will be accessible on a website and we will share plant material with interested researchers. In parallel, our collaborators are assessing the societal acceptance of genetically modified oranges. The willingness of consumers to purchase these oranges affects the growers’ interest to cultivate these trees commercially. This research reflects a need to consider GMO’s for agriculture in the wake of climate change, disease, and other threats to food production. This project is supported by award # 2020-70029-33160 from the National Institute of Food and Agriculture/U.S. Department of Agriculture.

Establishing a glioblastoma mouse model for evaluating the efficacy of novel therapies

J. V. Doorn, R. Sundaram, A. Mange, J. Vasquez, R. Bindra

Glioblastoma (GBM), a devastating disease, is the most common and aggressive form of primary brain cancer in adults. Presently there is an urgent need to develop better treatments for patients. One frequent GBM

mutation is in the IDH gene. In IDH-mutant cancers, one of the most important tools for repairing damaged DNA—the homologous-recombination (HR) pathway—is weakened. This makes IDH-mutant cancer cells more reliant on alternative DNA repair tools. PARP inhibitor (PARPi) drugs work by weakening several such alternative DNA repair tools, specifically the non-homologous-end-joining (NHEJ) and base-excision-repair (BER) pathways. PARPi-treated IDH-mutant cells must operate with simultaneously weakened HR, NHEJ, and BER DNA repair pathways, making them especially vulnerable to constantly-occurring DNA damage. Consequently, PARPi therapy is particularly effective against these cells. Normally when DNA is damaged, the ATR protein pauses cellular growth, giving the cell time to repair itself. When ATR is suppressed—for instance, with an ATR inhibitor (ATRi)—cells are forced to replicate with mangled DNA. Combined PARPi-ATRi treatment of IDH-mutant cells is expected to be effective. While PARPi accumulates unrepaired DNA damage, ATRi unpauses cell growth, forcing cells to divide and grow with damaged DNA. This in turn leads to cell death and tumor shrinkage. We plan to validate this combination therapy in IDH1-mutant glioblastoma. To this end, we will develop a mouse model of GBM by injecting IDH-mutant GBM cells into the brain and evaluating over time tumor size and overall survival, with and without treatment. Furthermore, we plan to assess specific effects of therapy on cells grown in dishes. Our findings will help determine whether PARPi and ATRi combination therapy will be effective in treating human patients with IDH1-mutant glioblastoma. These data can support the rationale for a clinical trial. If successful, this could result in an improved prognosis for patients with glioblastoma. This research is supported by an NIH Research Project Grant (R01), the Harold Amos Faculty Development Program, and the Vernon W. Lippard Summer Fellowship Fund.

Heat Inactivation of SARS-CoV2 in Saliva-based COVID-19 Diagnostic Testing

N. Vega, O.M. Allicock, D. Yolda-Carr, ..., A.L. Wyllie

Introduction: Robust SARS-CoV-2 surveillance remains key for countries continuing to face outbreaks, for communities that are starting to reopen and for understanding the long term efficacy of current vaccines. To minimize the risk of transmission during sample collection and processing, a viral inactivation step is necessary. While inactivation buffers are available for transporting saliva samples, these can dilute the sample, reducing the sensitivity of the test. We investigate the effect of heat-pretreatment on the sensitivity for SARS-CoV-2 detection in saliva samples. Method: We modified the SalivaDirect protocol to include a heat-pretreatment step (95℃ for 30 minutes, 95℃ for 5 minutes and 65℃ for 15 minutes, with and without a proteinase K step). These conditions were selected based on scientific literature demonstrating inactivation of SARS-CoV-2 in these conditions. To evaluate the 6 modified protocols, we processed de-identified saliva samples from confirmed COVID-19 positive individuals using these protocols and compared the sensitivity of SARS-CoV-2 detection by qRT-PCR. Results: For five of the six protocols tested, no significant difference was found when each limit of detection was compared to that of SalivaDirect. When tested on de-identified saliva samples from confirmed COVID-19 positive individuals, all of the conditions produced comparable virus detection to the standard SalivaDirect procedure. The absence of proteinase K did not negatively affect the sensitivity of the assay. Conclusion and general impact: The addition of multiple heat pretreatment options to the SalivaDirect protocol has increased the accessibility of this cost-effective SARS-CoV-2 test as it gives diagnostic laboratories the flexibility to implement the protocol which best suits their preexisting safety protocols. Many thanks to Orchid Allicock and Devyn Yolda-Carr for being supportive and informative resources as I adapted to this lab and dove into this project.

The effect of anti-MMP-9 as chemoattractant and its protentional role as adjuvant modulator of the immune response post cryoablation in a mouse model of liver cancer

A. Shewarega, J. Santana, A. Berz, D. Nam, V. Kahl, J. Theysohn, D. Madoff, A. Wetter, J. Chapiro

Introduction: The immunosuppressive tumor microenvironment (TME) remains a barrier to current immuno-oncologic therapies against hepatocellular carcinoma (HCC), which require the expansion of tumor antigen-specific T-cells into the TME and the removal of inhibitory enzymes, such as matrix metalloproteinase 9 (MMP-9), that limit T-cells responses. MMP-9 may antagonize T cell-mediated anti-tumor responses in tumors by increasing the recruitment of myeloid-derived suppressor cells to the tumor. Inhibition of MMP activity through the broad-spectrum MMP inhibitor Batimastat (BB-94) has shown to prevent tumor progression in animal models. Previous studies have also indicated that insufficient ablation of HCC could induce over-expression of MMP-9. Unlike other ablative therapies, cryoablation is thought to be particularly effective at generating anti-tumor immunity and eliciting systemic immune responses. Therefore, coalition with immunotherapy regimens, such as MMP-9 inhibition (MMPI), to boost and sustain the immune responses appears promising. Methods: To study the effects of MMPI and cryoablation on HCC in the context of a fully functional immune system, we use the Balb/C (Tib-75 syngeneic) orthotopic mouse model of HCC. Mice will be treated with BB-94 only, cryoablation only, and cryoablation plus BB-94. To identify the cellular sources of MMP-9 within the tumor, we assess MMP-9 expression and serial co-stainings with the macrophage marker CD68 and the CD206 marker for M2-polarized macrophages via immunohistochemistry. For quantitative comparison of CD8+ T cell invasion in the HCC TME, we use flow cytometry. Conclusion: This study aims to demonstrate that MMPI alleviates CD8+ T cell immunosuppression and enhances tumor immunotherapeutic effect and to prove that targeting the MMP process could help to increase overall therapeutic efficacy after cryoablation by amplifying the anti-tumor immune responses. General Impact: This project will lend greater insight into the immuno-modulatory effects of cryoablation and MMPI in HCC and offer a better understanding of how the TME modulates anti-tumor immunity.

Myeloproliferative Leukemia Virus Protein (MPL) regulates eosinophil trafficking in asthma

E. Bundschuh, A. Korde, J. Pober, A. Gonzalez, S. Takyar

Intro: Asthma is the most common allergic airway disorder that affects 236 million people worldwide and causes 461,000 deaths annually. Eosinophils mediate some of the injurious effects of allergic inflammation in airway tissues. Therefore, mechanisms affecting eosinophil entry into tissue may be useful therapeutic targets in asthma. Our previous work suggested that myeloproliferative leukemia virus protein (MPL) in the endothelium, the inner-most layer of cells in blood vessels, regulates the recruitment of eosinophils into inflamed lung tissue. Here we will examine the role of MPL in asthma patients and an animal model of asthma. Methods: We have collected sinonasal tissue samples from asthma patients treated at Yale New Haven Hospital. We have measured the levels of endothelial MPL by double staining for MPL and von-Willebrand factor, an endothelial marker. We also double stained for MPL and eosinophil peroxidase, an eosinophil marker, to measure eosinophil recruitment. The statistical analysis was done using GraphPad Prism 9. Asthma was modeled in mice by sensitization and boosting with ovalbumin (OVA) protein. To knockdown levels of MPL in mice, we delivered a small-interfering RNA. Results: We found that MPL is present in the airway endothelium. We will assess the correlation between the MPL levels in endothelial

cells, disease severity in patients, and the level of eosinophils in their blood and sinonasal tissues. Knockdown of MPL in the OVA model decreased the level of eosinophil recruitment in lung tissue by more than 50%. Conclusion/General Impact: Our findings suggest that endothelial MPL controls the recruitment of eosinophils into inflamed tissue and therefore is an important mediator of asthma severity. Altering levels of MPL in the endothelium may get rid of the damaging effects of eosinophils in lung tissues. This provides a promising and novel area for therapies for patients who do not respond to existing treatment options.

Developing a spatial multiomics technology with application to liver disease

K. Lapenta, X. *Yang*

Liver disease is a prevalent and rapidly growing burden on public health, currently affecting over 1.5 billion globally and increasingly fueled by rising rates of obesity and diabetes. Initial hepatocellular damage leads to chronic inflammation and fibrosis and can progress to cirrhosis and hepatocellular carcinoma in common conditions such as nonalcoholic fatty liver disease, alcoholic liver disease, and hepatitis B and C. Spatial and metabolic zonation are created across hexagonal liver lobules by hepatocytes lining the porto-central axis and directional blood flow forming gradients of oxygen and nutrients, respectively. This spatiotemporal organization is fundamental in homeostatic liver functioning and is known to be perturbed throughout pathogenesis of chronic liver diseases via fluctuation in tissue architecture and cell function. Single cell transcriptomics have illuminated dynamic changes in hepatocytes and resident liver immune cell populations throughout liver disease progression but lack important spatiotemporal context due to the disruptive nature of cell isolation. Dr. Rong Fan's lab group at Yale has recently developed deterministic barcoding in tissue for spatial omics sequencing (DBiT-seq) technology, utilizing crossflow DNA barcoding to co-map the mRNA transcriptome and proteome on a fixed tissue slide via next generation sequencing. This technique has produced incredible quality of gene expression and spatial resolution on mouse embryo tissues but has yet to be tested on diverse tissue compositions. We plan to apply this technology to human and mouse progressive liver disease specimens, and optimize the protocol as needed for altered tissue content such as increased fat and collagen deposition. Successful application of this technique to tissues from diseased livers will provide instrumental information on how spatiotemporal dynamics of resident liver cells regulate pathogenesis of liver diseases. This multi-omics approach presents a profound opportunity for new insight into chronic liver disease, and valuable potential for expansion of this technology. We would like to thank Dr. Rong Fan's group for the development and use of DBiT-seq technology; namely Archie Enniful, Graham Su, Xiaoyu Qin, Yanxiang Den, and Rong Fan for their collaboration.

COVID-19 in Connecticut Colleges and Universities During the 2020-2021 Academic Year: Contact, Testing, and Cases.

O. Schultes, *V. Clark*, *A.D. Paltiel*, *M. Cartter*, *L. Sosa*, *F. Crawford*

Introduction: During the 2020-2021 academic year, many institutions of higher education reopened to residential students while pursuing strategies to mitigate the risk of SARS-CoV-2 transmission on campus. Reopening guidance emphasized viral or antigen testing for residential students and social distancing measures to reduce the frequency of close interpersonal contact. In this paper, we study the patterns of COVID-19 testing, cases, and social contact in 18 residential college and university campuses in Connecticut. Methods: We compare institutions' 2020-2021 COVID-19 plans, submitted to the Connecticut Department of Public Health, with observed contact rates and COVID-19 outcomes throughout the academic year. Data on weekly residential

student testing and case data, residential enrollment, and move-in dates were provided by universities. The contact metric was derived from cell phone data measuring the probability of contact (i.e., interaction within 6 feet for more than 15 minutes) within each census block group. Results: In census block groups containing residence halls, fall student move-in resulted in a 450% (95% CI 367%-564%) increase in average contact, and spring move-in resulted in a 523% (417%-656%) increase in contact. Average testing rates per residential student per week ranged from 0.19 to 2.13 during fall and 0.42 to 1.86 during spring. During the entire academic year, only four institutions tested residential students more than once per week on average. Institutions with high rates of testing had lower COVID-19 case rates over both semesters (p=0.048). Residential student case rates were associated with higher case rates in the surrounding town (p<0.001). Conclusion: The results suggest that campus outbreaks among residential students can be avoided or mitigated by frequent testing and social distancing measures. Impact: Vaccination rates among residential students and surrounding communities may determine the necessary scale of residential testing programs and social distancing measures during the 2021-2022 academic year. We are grateful to Jacqueline Barbieri, Maciej Boni, Jessica Brockmeyer, Jared Campbell, Samantha Dean, Alexandra Edmundson, Deidre Gifford, Edward H. Kaplan, Albert I. Ko, Richard Levin, Olga Morozova, Alice Pritchard, Maura Provencher, William Shea, Suzanne Onorato, Tom Valleau, and Jennifer Widness.

Impact of temporary storage conditions on the viability of Streptococcus pneumoniae in saliva

P. Waghela, A. *Aklilu*, Y. *Yamamoto*, A. *Biswas*, *F. Calderon*, *F.P. Wilson*

Background: Nasopharyngeal swabs are most commonly used for Streptococcus pneumoniae carriage detection. Previous studies demonstrate that saliva samples can be more sensitive than swabs, however, there is little data available about the transport and laboratory storage conditions necessary for optimal detection. Our objective was to determine the optimal storage conditions for saliva samples for S. pneumoniae detection. Methods: To investigate the effect of freeze-thaw on S. pneumoniae viability in saliva, we spiked previously collected saliva samples with different serotypes (6C, 5, 11A, 3, 8, 7F, 15B, and 23F) at 1,000 and 10,000 CFU/uL and subjected these samples to 3 freeze-thaw cycles at -20℃ and -80℃. The spiked samples were also used to investigate the stability of S. pneumoniae at room temperature and 4℃over a period of 72 hours. The samples were then culture enriched followed by DNA extraction and a qPCR detection assay. Results: We detected pneumococcus at -80℃ across all serotypes after cycle 1 but many of the serotypes were not detected by cycle 2. We were able to detect all serotypes after 48 and 72 hours at room temperature and 4℃ respectively. Conclusion: Saliva samples can potentially be stored at room temperature or 4℃ temporarily without a significant loss of sensitivity for molecular S. pneumoniae detection. This makes saliva particularly useful as a sample type in pneumococcal carriage studies conducted in remote or low-resource settings. Thank you to Dr. F. Perry Wilson, my PI, for the opportunity to work on this incredible work. Thank you to my friends and family for their everlasting love and continued support.

Variations in fat metabolism predict outcome in murine models of sepsis.

M. Gao, *K. Israni-Winger*, *M. Nguyen*, *A. Wang*

N/A

Session 2 Program Abstracts

Effort-based decision making behavior is mediated by muscarinic acetylcholine receptors in the brain dopamine reward pathway

N. Kebede, E.J. Nunes, J. Haight, D. Foster, J. Conn, N.A. Addy

Introduction: Impairments in effort-based decision making behavior are common symptoms of depressive disorders and are often treatment resistant. Dopamine release in a reward pathway region, the nucleus accumbens, is implicated in regulating effort-choice behavior. Signaling of the neurotransmitter, acetylcholine, via muscarinic acetylcholine receptors, is critical for the regulation of dopamine release and has been shown to mediate depression-like behavior. The role of specific muscarinic receptor subtypes on effort-choice behavior, however, remains unknown. We hypothesize that the M5 subtype mediates effort-choice impairments via dopamine release in the nucleus accumbens. Methods: Effort-choice behavior was modeled using an operant paradigm where adult male and female rats were allowed to press for a more preferred food or consume freely-available food. Their effort-choice behavior was measured following administration of either an M5 inhibitor, to isolate M5 receptor function, or the drug physostigmine, to manipulate acetylcholine levels. To evaluate whether effort-related behavior is mediated by dopamine in the nucleus accumbens, dopamine activity was measured pre- and post-drug administration using a technique called fast-scan cyclic voltammetry. Results: Increasing acetylcholine levels decreased preference for the high effort, high reward option in both male and female rats, modeling a depressive-like behavior. Inhibiting M5 receptor activity, however, had no effect on effort-choice behavior. Interestingly, inhibiting M5 activity in the presence of increased acetylcholine attenuated the effort-choice impairment observed in the presence of increased acetylcholine in both male and female rats and increased nucleus accumbens dopamine release in male rats. Conclusion: Increased acetylcholine promotes effort-choice impairments in rats. Inhibition of M5 receptor activity in the presence of increased acetylcholine attenuates these impairments and increases dopamine release in the nucleus accumbens with no effect on its own. These data reveal M5 receptors to be important mediators of effort-related behavior and key pharmacological targets in treating effort-choice impairments present in depressive disorders. I would like to thank my mentor, Dr. Eric J. Nunes for helping conduct the behavioral and electrochemical experiments. I would also like to thank my supervisor, Dr. Nii A. Addy for his mentorship and guidance throughout my research. Lastly, I would like to acknowledge my colleagues in the Addy lab for their support and friendship.

Multimodal Interrogation of Statistical Learning and Episodic Memory in Epilepsy

A. Aljishi, D.M. Huberdeau, L. Lamsam, B.E. Sherman, J.T. King, A. Sivaraju, N.B. Turk-Browne, E.C. Damisah

Introduction: Memory impairment is a direct and leading cause of morbidity in all forms of epilepsy. Prior human studies suggest that Episodic memory, EM (i.e., ability to remember individual events), and Statistical learning, SL (extraction of patterns over time and space), depend on the medial temporal lobe (MTL) of the brain. However, it is not known whether SL and EM are equally affected in epilepsy. To address this gap, we developed novel behavioral methodologies combined with structural imaging and functional interrogation of different memory systems in epilepsy. Methods: 29 healthy controls (HC) and 38 epilepsy patients (EP) underwent computer-based behavioral testing designed to separately evaluate SL and EM. Manual segmentation of MTL was performed in EP to obtain volumes. In a subset of EP undergoing intracranial recording for seizure onset

localization, 1-Hz direct electrical brain stimulation (DES) designed to temporarily inactivate function in the MTL was implemented during behavioral testing. Results: Seizure frequency was the most important predictor of SL with EP where patients with poorly controlled seizures performed worse in SL compared to HC and EP with well-controlled seizures although their EM scores were comparable to HC. Consistent with prior literature, anti-seizure medications and MTL lesions affected EM score. Volumetric analysis of MTL demonstrated a correlation between SL performance and right MTL volumes in EP. Lastly, 1-Hz transient DES of the hippocampus during the learning phase of the SL task led to impaired recall performance. Conclusion: SL is a hippocampal-dependent learning system affected by seizure severity and right MTL volumes. Compared to EM, SL was a better predictor of cognitive function in epilepsy. General Impact: Investigating SL and EM may lead to a more comprehensive understanding of memory function in epilepsy, and provides an assortment of patient-centered memory-related endpoints used to evaluate the efficacy of novel treatments. This work is supported by the Kavli Institute for Neuroscience at Yale, Yale Center for Clinical Investigation (YCCI), and NeuroNext Fellowship

Tech for good: A scale for healthy technology use in adolescents

V. Tan, *S. McFarland*, *R. Baumsteiger*

Today's adolescents spend an unprecedented amount of time using technology: in 2019, adolescents spent an average of seven hours of screentime per day solely for entertainment purposes (Rideout & Robb, 2019). Emerging research in the field has investigated how the use of digital technologies may be associated with poor psychological well-being in adolescents, (Orben & Pzybylski, 2019). However, the results have been inconsistent and the field calls for a more detailed examination of adolescents' engagement with technologies. In addition, there has been growing interest in exploring the role digital technologies may play in supporting adolescents' health and well-being (Jensen et al., 2019; Orben, 2020; Vuorre et al., 2021). In light of this, the present study sought to create and validate a new measure of healthy technology use in adolescents. Seven domains of interest were included in the scale: online citizenship, tech anxiety, problematic internet use, tech safety, tech preparedness, technology and academics, and online social connectedness. These domains were inspired by the modules provided in the Facebook Digital literacy Library and included with the purpose of capturing a more holistic view of tech use in adolescents. Study 1 (N=4773 students) established a six-factor, 28-item scale using exploratory factor analysis, but with varying internal reliability. Based on the results of Study 1, new items were created for the subscales with lower internal reliability; surveys retaining the previous items and the additional items were distributed in Study 2 (N = 3567). A principal components analysis was conducted to investigate which of the newly added items loaded onto the proposed factors, and the subscale reliabilities (α) ranged from 0.625 to 0.822. Further research and practice can apply the healthy adolescent technology use scale to better understand the impact of digital technologies on adolescents' psychological well-being. We thank Dr Jessica Hoffmann for her guidance as well as her work in creating items used in the scale. We also thank Jenny Seibyl for her support. This study was supported by the Susan Crown Exchange Grant.

Characterizing people's responses to the US state governments' reactions in the COVID-19 pandemic

E. Wertheimer, *R. Jia*, *M. Chawla*, *O. Duek*, *G. Fuhrman-Alpert*, *R. Larisch*, *I. Levy*

The events of 2020, particularly the presidential election and the arrival of COVID-19 to the United States, highlighted deep schisms within the American collective. The present research investigated constituents' reactions to their state and federal governments' responses to the COVID-19 pandemic. From the end of March

until early May of 2020, 444 participants recruited via the virtual crowdsourcing labor force, Amazon Mechanical Turk (AMT), completed a comprehensive, longitudinal survey probing their demographic information and in-depth attitudes regarding their understanding of, and reaction to, the pandemic. They also completed two experimental tasks adapted from behavioral economics, one standard and one novel, designed to measure participants' risk and ambiguity attitudes in the monetary and medical domains, respectively. Publicly available data regarding population and COVID-19 tests, deaths, and hospitalizations were obtained as additional data points. These publicly available data were analyzed in conjunction with the survey measures. General linear models showed that participants' conception of the state of residence's reaction to the COVID-19 pandemic depended on the respondents' age ($p < 0.05$), political typology ($p < 0.001$), and their state's COVID-related death rate at the time of the survey ($p < 0.05$). Specifically, older participants, conservative participants, and participants residing in states with high death rates considered their state to be "overreacting" to the COVID-19 pandemic. Future research relating task-derived decision attitudes to participants' individual reactions to the pandemic is warranted given the continued socio-political and pandemic-related polarization in the United States.

Developing a High-throughput Brain Activity Mapping Pipeline to Analyze ASD Risk Gene Function

U. Neelakantan, *D. Jin, T. Chen, H. Weinschutz Mendes, S.E. Fitzpatrick, M. Carlson, K. Enriquez, A. Pruitt, I. Nwabudike, X. Papademetris, E.J. Hoffman*

Introduction: Autism spectrum disorder (ASD) is a clinically and genetically heterogeneous neurodevelopmental disorder characterized by deficits in social communication and repetitive behaviors. Recently, whole exome sequencing studies of individuals with ASD and their families have identified >100 high confidence ASD risk (hcASD) genes. However, our understanding of how these genes function in the developing brain is limited, yielding a need to develop high-throughput in vivo approaches for screening hcASD gene function. Zebrafish are an optimal system for investigating this relationship because they allow direct visualization of whole-brain structure and activity at early neurodevelopmental stages. Using CRISPR/Cas9, we generated zebrafish mutants of 10 hcASD genes to investigate how loss of hcASD gene function affects brain activity and structure during early neurodevelopment. Methods: Here we present a high-throughput analysis pipeline that measures regional changes in brain volume and activity. Brain activity is measured by immunostaining larval zebrafish brains using antibodies to phosphorylated ERK (pERK), representing active neurons, and total ERK (tERK), representing all neurons. We quantify this activity by mapping confocal images onto a standard zebrafish reference brain and calculating the ratio of pERK/tERK using the Z-brain atlas, adapting a method developed by Randlett et al (2015). Using nonlinear registration, we visualize these calculations and identify candidate regions (ROI) from the Z-brain atlas based on altered size or neural activity. Conclusion: We automated this method utilizing High Performance Computing (HPC) Systems, which allowed us to analyze multiple mutant lines in parallel. Our pipeline is also optimized to include more checkpoints for error and is customizable to incorporate quantification of other neural activity markers such as GABAergic and glutamatergic neurons. General Impact: This high-throughput pipeline will help us uncover mechanisms underlying altered neurodevelopment in ASD and expand our understanding of how mutations in hcASD genes might lead to similar clinical presentations. I would like to thank the entire Hoffman lab specifically my mentor and PI, Ellen J. Hoffman and lab mates Sarah E. Fitzpatrick, Tianying Chen, Hellen Weinschutz Mendes, Marina Carlson, Kristen Enriquez, April Pruitt, and Ijeoma Nwabudike for the endless support, guidance, and efforts on this project. Additionally our collaborators David Jin and Xenophon Papademetris for making this pipeline possible.

The Recovery In Stroke Using Positive Airway Pressure (PAP) Study (RISE-UP)

E. Karagoz

When considered separately from heart disease, stroke is the third leading cause of death in the United States and the second leading cause of death worldwide. It is the leading cause of disability. New therapeutic approaches to help prevent stroke and improve functional disability post-stroke are needed. Previously, our team at the Yale School of Medicine showed that obstructive sleep apnea, a disorder in which breathing repeatedly stops and starts during sleep, significantly increases the risk for stroke and all-cause mortality independent of other stroke risk factors (Yaggi, NEJM, 2005). It remains unclear whether treating sleep apnea in the acute stroke setting may improve functional recovery post-stroke. Our research aims to determine whether positive airway pressure (PAP) treatment for obstructive sleep apnea (OSA) can improve post-stroke functional recovery. The Recovery in Stroke Using PAP Study (RISE-UP) is a randomized controlled trial consisting of 180 patients with acute ischemic stroke and moderate/severe OSA diagnosed by ambulatory polysomnography. We will compare PAP treatment with usual care over six months. The participants will be randomized into three groups: 60 patients into acute intervention (PAP therapy started within one week post-stroke), 60 patients into subacute intervention (PAP therapy started one month post-stroke), and 60 patients into the control group (usual care). The Modified Rankin Scale will be used as the primary outcome to assess post-stroke functional recovery at six months, along with several secondary outcome measures such as the NIH Stroke Scale. If the findings of our study show that PAP therapy for sleep apnea improves functional recovery post-stroke, this project can change clinical practice by implementing sleep testing and PAP therapy in the acute post-stroke period. The research discussed in this abstract is supported by the National Institute of Nursing Research (NINR) of the National Institutes of Health under award number 5R01NR018335-03. The PI of this study is Dr. Henry Klar Yaggi.

A Multimodal Cortical Atlas for Epilepsy Surgery and Function-Structure Hypotheses

T. Jafar, D. Spencer, H. Zaveri

Epilepsy surgery evaluation generates large quantities of multimodal data including imaging and electrophysiology. For research and clinical decision-making, it is critical to aggregate this data in a common space. Existing brain atlases lack an intuitive naming or parcellation scheme that provides easy cross-referencing of multimodal data. We define an atlas that addresses these issues in epilepsy surgery and serves as a visual database for a cohort of patients. We registered multimodal data from epilepsy patients onto our cortical brain atlas that is parcellated to reflect standard 1cm contact spacing of intracranial electrodes and defined relative to robust landmarks on the MNI152 brain. Structural 3TMRI images underwent linear/nonlinear registration, and 3D electrode coordinates were transformed to the atlas space, which was verified using Yale BioImageSuite software. Electrophysiology data on seizure onset and spread regions was mapped, and bipolar stimulation mapping for language was incorporated. A total of 3386 intracranial electrode contacts from 20 temporal-lobe epilepsy patients were transformed to the atlas: an average of 169 contacts per patient. The cohort was split in two: ten patients had a mesial-temporal onset of seizures, and ten had a neocortical onset. In the mesial-temporal group, the most common parcel of onset was HA (pes hippocampus) in 50% followed by HB (hippocampal body) in 40%. In a comparison of illustrative cases, a patient with onset in HB underwent an extended temporal resection of parcels involved at onset, first and second spread, and subsequently remained seizure-free at one-year. The second patient had spread outside of the resected neocortical parcels and failed surgery. We present an epilepsy surgery atlas for aggregating multimodal data. The atlas allows for

co-registration of multiple data sources onto the same visualization to allow for exact cross-referencing when used for clinical decision-making and research. As well as map and compare patient data for outcome correlation.

Does Rental Assistance Improve Mental Health? Insights from a Longitudinal Cohort Study

W. Denary, A. Fenelon, P. Schlesinger, J. Purtle, K.M. Blankenship, D.E. Keene

Almost half of renters in the United States are rent-burdened, meaning that they pay more than 30% of their income toward housing costs. Rental assistance through programs administered by the U.S. Department of Housing and Urban Development, alleviates these financial strains for around 5 million households. However, due to budgetary constraints, fewer than one in four eligible households actually receive this assistance and waitlists average two years nationally. Using longitudinal data from a cohort of 400 low-income adults living in New Haven, CT, this paper investigates how access to rental assistance affects mental health through two analytical methods that address selection into rental assistance. First, we performed a cross-sectional analysis to identify how psychological distress differs among those receiving and those on a waitlist for rental assistance. Second, we used a within-person fixed-effects analysis to compare changes in individuals following entry into rental assistance. We find that those receiving rental assistance report significantly less psychological distress than those on waiting lists and that transitions into rental assistance are associated with statistically non-significant decreases in psychological distress. Our findings suggest that expanding rental assistance may be one potential step toward improving the mental health of low-income individuals in the United States. Funding for this study was provided by the National Institute of Mental Health and the National Institute of Allergy and Infectious Diseases (RO1MH110192 Kim M. Blankenship, Ph.D., Principal Investigator)and by the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK124500 Danya E. Keene, Ph.D., Principal Investigator). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Correlates of Pre-exposure Prophylaxis (PrEP) Use among Men who have Sex with Men (MSM) in Malaysia

W. Eger, A. Adaralegbe, A. Khati, S. Osborne, I. Azwa, J. Wickersham, R. Shrestha

Introduction: Pre-exposure prophylaxis (PrEP) for human immunodeficiency virus (HIV) is an effective public health intervention to prevent HIV among men who have sex with men (MSM). Although PrEP is included in Malaysia's National HIV Prevention Strategy, its use has not been widespread. Therefore, this project aims to explore sociodemographic and health-related behaviors associated with PrEP uptake among Malaysian MSM to further direct public health programming. Methods: A sample of 355 Malaysian MSM completed an online survey between June and July 2020. Participants were recruited using advertisements on geosocial networking apps for MSM (Grindr, Hornet) and social networking websites for the general population (Facebook). Descriptive and multivariate analyses were used to examine PrEP uptake within this population of MSM. Results: The sample was predominantly Malay (53.5%); had monthly incomes greater than RM 3,000 (approximately USD 730); and a tertiary level of education (84.5%). Less than one-fifth of participants (18.3%) had taken PrEP before completing the survey. In the multivariable analysis, using drugs prior to sexual intercourse (OR: 3.37; p=0.005), being diagnosed with a sexually transmitted infection (OR: 2.08; p=0.019), recent HIV testing (OR: 3.23; p=0.000), and disclosure of sexual orientation (OR: 1.85; p=0.042) were significantly associated with a greater likelihood of having taken PrEP in the past. Conclusions: PrEP uptake among Malaysian MSM is relatively low and markedly lower than previously hypothesized. Existing HIV prevention programs should be redefined to meet the needs of

MSM community members in Malaysia, with updates focused on providing care to individuals not currently engaged in traditional healthcare settings. General Impact: Prior to this study, there have been no assessments of factors associated with PrEP uptake among Malaysian MSM. This research will help develop public health programming by identifying specific avenues for implementing the most effective HIV prevention programs. This project is funded through the Fordham RETI Award, under the Principal Investigator Roman Shrestha, PhD, MPH

Optimizing an integrated rapid access program to HIV prevention and opioid agonist therapy in people who inject drugs through discussions with clinical and community stakeholders

J. Lee, *W. Eger*, *S. Osborne*, *A. Khati*

Background: Specific injection drug behaviors are fueling human immunodeficiency virus (HIV) acquisition among people who inject drugs (PWID). Preexposure prophylaxis (PrEP) has proven to be effective at combatting HIV; however, lack of access and awareness, negative provider attitudes, and structural barriers have led to poor uptake among PWID. We plan to optimize the development of an integrated rapid access to HIV prevention and opioid agonist therapy (OAT) program for people who inject drugs (iRaPID) by evaluating barriers for such a program and discussing potential solutions. Methods: Nominal Group Technique (NGT) was used to ascertain barriers and solutions for the implementation of the iRaPID program. Recordings and detailed notes collected during three NGT sessions with 17 clinical stakeholders and PWID were used to establish major themes. Results: The top three barriers among PWID to getting a same-day prescription for PrEP and OAT were: (1) lack of insurance; (2) lack of transportation; and (3) health behaviors such as lack of motivation. Among clinical stakeholders, the three greatest challenges were: (1) shortage of providers to prescribe medications; (2) unreliability of the target population; and (3) program-related logistics like coordination between prescribers and eligible medication distributors. Potential solutions identified by the clinical stakeholders and PWID included: (1) increasing access to transportation; (2) streamlined prescribing and communication mechanisms; and (3) direct delivery of medication. Conclusion and General Impact: Same-day access to PrEP and OAT was viewed favorably by clinical stakeholders and PWID as a mechanism for streamlining the process for medication retrieval, thus improving PrEP uptake and HIV prevention. Recurrent challenges among clinical stakeholders and PWID for the implementation of the iRaPID program included transportation barriers and lifestyle-related challenges. Solutions to these barriers could include incentivization via bus passes or the coordination of medication delivery by eligible pharmacies. NIH NIDA.

Session 3 Program Abstracts

Development of Radiolabeled Antibodies to Track Cytotoxic T-Cells and Tumor-Associated Macrophages Concurrently by SPECT Imaging

E. Belitzky, *M. Liu*, *S. Lee*, *F. Bergara*, *S. Thorn*, *C. Liu*, *A. Sinusas*, *M. Bosenberg*, *H. Kluger*, *A. Wu*, *B. Marquez-Nostra*

Introduction: Non-invasive detection of biomarkers has promise in longitudinal tracking of immune cells towards predicting individual tumor response to immunotherapy. Single Photon Emission Computed Tomography (SPECT) can image multiple biomarkers concurrently with radiolabeled antibodies given they are attached to radioisotopes that emit distinct gamma energy signatures. The purpose of this project is to develop

the radiosynthesis and characterize the binding properties of SPECT agents for cytotoxic T-cells and tumor-associated macrophages (TAMs) for future application tracking these immune cells in animal models of cancer in response to immunotherapy. Methods: Anti-CD8 cys diabody and anti-CD68 IgG bind to T-cells and TAMs, respectively. SPECT agents for CD8 and CD68 were designed with Tc-99m and In-111 radioisotopes, respectively to match biological half-lives of the antibody with physical half-lives of the radioisotope. Conjugation of antibodies to chelators and radiolabeling was optimized and assessed with size-exclusion and radioactive thin-layer chromatography to determine radiochemical yield and purity. Binding affinity was determined by a saturation assay. Biodistribution studies with the anti-CD8 agent using non-tumor bearing mice were performed to quantify uptake of the agent in various organs. Results: Radiochemical yield was greater than or equal to 95% for both agents. The anti-CD8 agent had 20% antibody aggregation and the anti-CD68 agent had 11% antibody aggregation. The binding affinity of the anti-CD68 agent was determined to have a dissociation constant (KD) of 6.758 nM. A biodistribution study of the anti-CD8 agent shows uptake in liver and kidneys for clearance and uptake in spleen and lymph nodes as expected in T-cell rich organs. Conclusion and General Impact: Results demonstrate successful production of both SPECT imaging agents. Determining potential response to immunotherapy and monitoring response over time using SPECT can identify responders and non-responders earlier during treatment to more effectively use immunotherapy to treat cancer and spare non-responders from unnecessary toxicity. This study was supported in part by the Yale Cancer Center Pilot grant.

Behaviour of adsorbed proteins on gold surface

P. Komorek, *E. Martin, B. Jachimska*

Introduction: Protein-surface interactions can lead to the formation of protein films with varied characteristics. The ability to control the properties of protein layers adsorbed on the surface is of particular importance to the designing of innovative biosensors, manufacturing new biomedical implants, and developing tissue engineering. Therefore in this study, the hydration, viscoelasticity, and hydrophobicity of lysozyme films, as well as the conformation of adsorbed lysozyme molecules on the gold surface were investigated in various conditions. Methods: Complementary measurements using Surface Plasmon Resonance and Quartz Crystal Microbalance allowed establishing hydration of lysozyme films on gold. The viscoelasticity and hydrophobicity of obtained layers were investigated using Quartz Crystal Microbalance and Contact Angle measurements, respectively. The changes in lysozyme conformation as a result of interactions with the gold surface were established based on Circular Dichroism and Infrared Spectroscopy. Results: The hydration of adsorbed protein layers is dependent on the effective charge of protein and surface coverage. Higher surface coverage translates into a decrease in water content to 60% and lower hydrophobicity of lysozyme monolayers on gold. However, formation of bilayers is associated with the rapid rise in hydration up to 85%. Highly hydrated bilayers are more viscoelastic compared to monolayers, which was confirmed by the determination of shear elasticity and viscosity modules. Parallel measurements in bulk solution and adsorbed state showed that lysozyme is stable in bulk independently on pH, while interactions with gold cause strong changes in protein structure in direction of β-turn and random coils formation, which intensified in higher pH. Conclusion and Impact: This study presents in detail protonation and surface coverage, along with the hydration, hydrophobicity, viscoelastic properties of the layers, and conformation of adsorbed lysozyme. We showed that the characteristic of obtained films can be controlled by environmental conditions during adsorption. These findings are especially crucial for designing new materials for biological applications. This work was partially supported by project NCN OPUS

2016/23/B/ST5/02788 and NCN PRELUDIUM 2020/37/N/ST4/02132, InterDokMed POWR.03.02.00-00-I013/16 and NAWA PPN/BIL/2018/1/00103.

Controlled Self Assembly of Microbial Nanowires into Ordered Lattices

G. Mandava, *M. Guberman-Pfeffer*, *P.J. Dahl*, *C. Shipps*, *S. Yalcin*, *Y. Gu*, *V. Srikanth*, *D.M. Shapiro*, *V.S. Batista*, *N.S. Malvankar*

N/A

Noise Analysis and Stochastic Modeling of EPICA Ice Core Climate Records

N. Keyes, *J.S. Wettlaufer*, *L. Giorgini*

The past 800,000 years of Earth's climate history encapsulated in the EPICA Dome-C ice core are characterized not only by eight glacial cycles, but also by noisy behavior at many time scales. To describe this noise behavior in EPICA's CO2, CH4, and dD temperature proxy data, we perform multifractal time-weighted detrended fluctuation analysis (MFTWDFA). This method successfully distinguishes red-noise and white-noise behavior below and above the 100,000-year timescale respectively in all three datasets. Based on this noise behavior, we model each time series as a one-dimensional white-noise Ornstein-Uhlenbeck process, and then simulate the time series with this model to confirm that it can reproduce the complex behavior of the data. Then, we extend this to two- and three-dimensional coupled models, in order to estimate the strength and direction of influence of the three time series on each other. Based on the linear couplings, we find that CO2 and dD influence each other and also CH4, while CH4 does not have much influence on CO2 or dD. Our approach demonstrates that stochastic-process models can represent noisy, complex paleoclimate time series in a simple way that gives insight into the relationships between climate variables. This modeling also lays the groundwork for investigation of stochastic resonance and critical transitions in noisy climate data. Many thanks to Professor John Wettlaufer for his thesis advisorship and support for the past two years, and to Ludovico Giorgini for his mentorship. This work was funded by the Von Damm fellowship from the Earth & Planetary Sciences Department, and will be continued next year through funding from Professor Wettlaufer.

An Automated, Open-Source Program to Standardize Acute Kidney Injury Definition from Timestamped Creatinine Data

I. Saran, *A. Aklilu*, *Y. Yamamoto*, *A. Biswas*, *F. Calderon*, *F.P. Wilson*

Introduction: Due to the complex etiology of its presentation, AKI has been defined in a variety of ways. A standardized definition is necessary in order to make accurate comparisons across captured patient populations, to refine clinical understanding of the syndrome for better prognosis, and to lower administrative costs associated with AKI. Methods: We seek to streamline the standardization of AKI by presenting the AKIFlagger, an open-source computational tool built in Python, R, and a web application which implements a standardized AKI definition based on KDIGO guidelines while allowing for variational definition of historical baseline. We applied the AKIFlagger to a dataset of patients hospitalized with COVID-19 with three functional approaches to defining AKI: (1) a rolling-window definition, (2) a historical baseline definition, and (3) imputation based on demographic information. Results: In our dataset, we demonstrate that subtle changes in definition - such as inclusion of imputed baseline creatinine values - can have a large impact on which patient populations are captured. Compared to the rolling window KDIGO guidelines, using a “historical baseline” definition of

creatinine and allowing for imputed historical baseline creatinine values increases the size of captured patient populations by 20.7% and 57.1%, respectively. We characterize the predictive value of the different definitions by determining the sensitivity and specificity for stage progression and progression to death or dialysis. The approaches span sensitivities from 0.71 to 0.85 and specificities from 0.62 to 0.76 for progression to death. General Impact: Subtle differences in the definition of AKI can lead to drastic differences in which patient populations are captured by the definition. A standard definition of AKI is necessary for the field to accurately advance both clinical and basic science research. This standardized tool can be used by researchers to ensure definitions are uniform across studies. Thank you to Dr. F. Perry Wilson, my PI, for the opportunity to work on this incredible work. Thank you to my friends and family for their everlasting love and continued support. Thank you to Dr. F. Perry Wilson, my PI, for the opportunity to work on this incredible work. Thank you to my friends and family for their everlasting love and continued support.

Session 1 Poster Program Abstracts

Poster #1

Psilocybin Induces Structural Plasticity and Behavioral Resilience to Stress

I. Gregg, L. Shao, C. Liao, N.K. Savalia, K. Delagarza, A.C. Kwan

Introduction: Psilocybin is a serotonergic psychedelic and a promising compound for treating depression in humans. However, the neurobiological mechanisms of psilocybin are poorly understood. Here we characterize the behavioral and neurological effects of a single dose of psilocybin. Methods: We tested 82 C57BL/6J mice at five doses of psilocybin and counted head-twitch responses for ten minutes following injection. We pretreated mice with 5-HT2A receptor antagonist, ketanserin (1 mg/kg), and repeated the procedure (1 mg/kg psilocybin; n=10). Using confocal microscopy, we imaged dendrites in the medial frontal cortex of Thy1¬-GFP-M¬ mice 24 hours after an injection of psilocybin or an equivalent amount of saline (1 mg/kg; n=12 mice). We blindly scored the dendritic spines in the Cg1/M2, M1, and PrL/IL subregions for density, protrusion length, and head width. Using two-photon microscopy, we assayed spine turnover by imaging the medial frontal cortex at seven timepoints (n=12). We tested psilocybin's effects on behavioral resilience in the learned helplessness paradigm compared to saline (negative control) and ketamine (positive control; n=68). We examined the role of post-administration context in mediating these behavioral effects (n=57). Results: Psilocybin evoked robust head-twitch responses at and above 1 mg/kg. Head-twitches were fully blocked by ketanserin. A single dose of psilocybin led to increased spine density and spine head diameter in specific subregions of the cortex. Some of the newly formed spines persisted for 34 days. Psilocybin ameliorated the learned helplessness state, but this result wasn't significant between groups. Post-administration context did not have a discernable effect on behavior. Conclusions: A single dose of psilocybin acts through the 5-HT2A receptor to produce head-twitch responses, induces persistent changes to the dendritic architecture of the mouse medial frontal cortex, and increases resilience to stress. General Impact: These results provide insights into the biological mechanisms underlying the psilocybin's therapeutic actions. This work was supported by the Yale Center for Psychedelic Science, NIH/NINDS training grant T32NS041228 (C.L.), and NIH/NIGMS Medical Scientist Training grant T32GM007205 (N.K.S.). We thank the Yale Center for Advanced Light Microscopy Facility for their assistance with confocal imaging, supported in part via NIH grant S10OD023598.

Poster #3

A2aR expression in astrocytes contributes to multiple sclerosis progression

M. Mansoor, C.H. Lo, G. Ponath, S. Toro, C. Chen, S. Bhandarkar, M. Crane, C. Park, D. Dijk, L. Airas and D. Pitt

Adenosine 2a receptors (A2aRs) are prevalent in the CNS and have been implicated in several diseases such as AD, PD and ALS. Currently, the role of A2aRs in multiple sclerosis (MS) progression is unknown. We employed PET imaging of MS patients, single-cell multiplexed analysis and cell assays in MS lesions to elucidate the role of A2aR in MS progression. We found that A2aR expression is higher in the normal appearing white matter (NAWM) of secondary progressive MS (SPMS) patients relative to relapsing-remitting MS (RRMS) patients; furthermore, we identified two astrocyte A2aR^high populations localized to the rim and NAWM of MS lesions. In addition, in vitro experiments in human fetal astrocytes revealed A2aR signaling causes downregulation of cell adhesion proteins and disrupts astrocytic connectivity pointing towards higher infiltration of lymphocytes into the brain parenchyma exacerbating disease progression. Our findings suggest that A2aR signaling in SPMS patients

worsen disease progression and could be a potential target for therapeutic intervention to contain disease progression. I would like to thank the Pitt Lab, my parents, my sisters and my friends.

Poster #5

Domain General Processes for Interactive Touch

T. Buck, C. DiCocco, J. Cuzzocreo, A. Noah, X. Zhang, & J. Hirsch

The nexus model of social processing proposes that the right temporal parietal junction, rTPJ, serves as a neural hub for social processes. If the region is domain general, then interactive handshakes would also activate rTPJ contrary to expectations of known contralateral sensory processes. Right- and left-hand handshakes with real vs simulated hands during fNIRS neuroimaging were consistent with a domain general model. This research was partially supported by the National Institute of Mental Health of the National Institutes of Health under award numbers 1R01MH111629 (PIs JH and JM); R01MH107513 (PI JH); 1R01MH119430 (PI JH).

Poster #7

Neural mechanisms supporting memories for alcohol-related events

B. Harris, E. Goldfarb

Memory plays a key role in addiction development and relapse. Crucially, memories of a single event can be broken down into items (individual parts of a memory) and contexts (integrations of different elements of a memory). Recent work indicates that individuals with alcohol use disorder are biased to remember alcohol-related contexts, while disproportionately forgetting items. However, the neural mechanisms underlying the formation of distinct components of alcohol-related memories are unclear. We hypothesize that alcohol-related events will be remembered differently from neutral events in social drinkers, and that alcohol-related memories will be associated with stronger BOLD responses in the dorsolateral striatum and perirhinal cortex (items) and hippocampus (contexts). We first developed and validated a set of 320 matched alcohol-related and neutral handheld object stimuli for use in a two-day memory task. Next, we enrolled 8 participants (mean age = 29.25, 63% female, mean AUDIT = 5.50, target N = 28) in an ongoing fMRI experiment. During the study, participants encoded 160 associations between pairs of objects (50% alcohol, 50% neutral) and neutral scenes. 24 hours later, memories for items (object recognition) and contexts (cued recognition of scenes associated with objects) were assessed. Preliminary analyses indicate a slight memory bias for neutral items ($p = .11$) and "gist"-level memory for alcohol-related contexts ($p < .01$). We are currently analyzing fMRI data to identify regions in which BOLD signal at encoding supports successful item and context memory for alcohol compared to neutral events (remembered vs forgotten for alcohol vs neutral). This research has the potential to uncover the neural mechanisms supporting memories associated with addiction and may help guide treatment design. Future research should strive to elucidate these mechanisms in clinical and non-drinking participant populations. Thank you Karen Martin for helping run all of our scans. And thank you to Shruti Parthasarathy, Grace Larrabee, and Darlene Gomez for all your help cultivating the stimulus set.

Poster #9

Investigating changes in neurological activity, counterregulatory hormones, and brain-glucose transport in hypoglycemia unaware patients

J. Deajon-Jackson, J.J. Hwang

Hypoglycemia is the major limiting factor for optimal glycemic control amongst patients with type 1 Diabetes Mellitus (T1DM). Frequent exposure to hypoglycemia can result in the development of hypoglycemia unawareness. Though past research has identified differences in brain activity in hypoglycemia unaware T1DM patients, the exact mechanisms remain unclear and may involve changes in the interplay of neurological activity, levels of metabolites and counterregulatory hormones, and brain-glucose transport. To fill this gap, we plan to utilize magnetic resonance spectroscopy (MRS) and functional magnetic resonance imaging (fMRI) to investigate 3 groups of subjects (healthy, non-diabetic control (HC), T1DM patients with hypoglycemic unawareness (T1DM-Unaware), and T1DM patients without hypoglycemia unawareness (T1DM-Aware). Participants will undergo 2 separate scanning sessions: 1) 13C magnetic resonance spectroscopy (MRS) scanning used to measure rates of glucose transport in the brain; 2) functional connectivity MRI scanning used to identify changes in brain connectivity patterns during 3 tasks designed to assess reward learning (card-guessing task), working memory (n-back task), and attention (gradCPT task). This study aims to improve knowledge on the physiological differences between T1DM-Unaware and T1DM-Aware patients, while investigating neurological differences that could explain the contrast in symptom sensitivity. By combining data from MRS and fMRI we can assess the connection between sensitivity to the symptoms of hypoglycemia, neurologic activity in response to decreased blood-glucose, and alterations in counterregulatory hormones and metabolites in the brain. Furthermore, a greater understanding of the neurological signs associated with hypoglycemia unawareness could provide a more definitive means of identifying future T1DM-Unaware patients. I would like to acknowledge Janice J. Hwang, Elizabeth Sanchez-Rangel, Charles Watt, and Mari-Lynet Knight for their help in understanding this project.

Poster #11

Developing an Adapted Experimental Pain Task for Therapeutic Assessment

L. Sullivan, *N. Fogelman*, *K. Smith*, *J. Schwartz*, *R. Sinha*

Broadening legalization of medical marijuana at the state level has allowed an increasing number of Americans access to prescribed marijuana for a number of medical conditions. Chronic pain is one such condition that increases use of medical marijuana products. In a protocol that assessed the safety of several acute tetrahydrocannabinol (THC) and cannabidiol (CBD) single doses, eight recreational marijuana users participated in six experimental sessions, once per week for six weeks. An Adapted Cold Pressor Task (ACPT) including an uncontrollable psychological stressor was conducted each session to compare the effects of hand immersion in ice-cold and warm water, where trials were repeated three times every session for both conditions. Physiologic, endocrine, and subjective pain and anxiety ratings were assessed for each trial. Results show highly significant effects of repeated cold versus warm water hand immersion on subjective pain ratings, anxiety, heart rate, blood pressure, and stress hormone cortisol levels (p's<.01). Findings indicate that the ACPT may be a valid task in assessing THC and CBD effects on pain and anxiety, as well as the biological responses to such pain. Many thanks are owed to Connecticut Pharmaceutical Solutions (CPS), and to the extensive work of Rajita Sinha, Keisha Smith, Nia Fogelman, and Julie Schwartz for making this project possible.

Poster #13

Assessing Epigenetic Aging in Individuals with Substance Use Disorders

A. Figueroa-Jiménez, *S. Nagamatsu*, *H. R. Kranzler*, *J. Gelernter*, *J. Montalvo-Ortiz*

Introduction: Changes in DNA methylation at specific cytosine-phosphate-guanine (CpG) sites occur with aging and can be used as an indicator of biological age. Epigenetic clocks can provide significant age-related metrics to predict heart disease, cancer, and mortality. Emerging data show that substance use disorders (SUDs) can lead to an acceleration in DNA methylation (DNAm) age contributing to premature death and age-related diseases. However, studies have mainly focused on smoking and alcohol-related traits; traits related to cocaine, cannabis, and opioids are less studied. Methods: Our study included 1,009 individuals from the Yale/Penn cohort, a collection of individuals with multiple SUDs and screened controls. The sample had a median age of 41∓11.6, 60% of females and with an ancestry distribution of 60% European and 40% African. SUDs were diagnosed with the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA), a polydiagnostic psychiatric assessment. SUDs that will be tested include nicotine (n=687, 68%), alcohol (n=649, 64%), cannabis (n=396, 39%), cocaine (n=634, 63%), and opioid dependence (n=379, 38%). Epigenetic aging was calculated for each individual based on peripheral blood profiled with the Illumina Infinium EPIC array. DNAm GrimAge, a novel and accurate epigenetic clock will be calculated using the online DNA Methylation Age Calculator tool. Association analysis will be conducted covarying for sex, age, ancestry, cell-type proportions and other SUD diagnoses in individuals with multiple SUDs. Results and Conclusion: Ongoing. General Impact: Accelerated epigenetic aging has been associated with smoking and alcohol traits. Less is known about the effects of other SUDs, particularly cannabis, cocaine, and opioid dependence, on epigenetic clocks. This will be the first well-powered study to use whole blood to evaluate epigenetic aging among individuals with different SUDs. This study will assess the association between SUDs and epigenetic age acceleration as a biomarker of mortality risk and age-related disorders.

Poster #15

The Impact of Trait Anxiety When Evaluative Pressure Is Increased

O. Baker, D. Feigley

Athletes and anxiety have been studied extensively within the sports psychology field; however, minimal research has focused on the subset of trait anxiety. This proposal will assess and identify the levels of trait anxiety that interfere with gymnasts' balance beam performance. Gymnasts high in trait anxiety are anticipated to have lower performance scores than individuals with low trait anxiety when placed in high-pressure conditions. Gymnasts with low trait anxiety should have smaller differences in performance scores when their scores are compared from the low-pressure to the high-pressure condition. To determine if the experimental procedure designed to induce perceived pressure is effective, all gymnasts will be assessed for their levels of state anxiety. Low and high trait anxiety gymnasts are predicted to show higher state anxiety when being evaluated by gymnastics judges. The researchers will conduct performance assessments, self-report surveys, and a 2x2 mixed design analysis of variance to determine if high levels of trait anxiety have debilitating effects on gymnasts' balance beam performance.

Poster #17

Exploring the Relationship Between Post Traumatic Stress Disorder, Depression, and the Reward System

R. Seidemann, O. Duek, I. Harpaz-Rotem

The effect of trauma on reward-system functioning is under-researched, and some theorize that observed deficits (anhedonia, emotional numbing) may be better explained by comorbidity with Major Depressive Disorder (MDD). This study aims to examine the relationship between post traumatic stress disorder (PTSD),

MDD, and the reward system. The study sample consisted of 118 veterans (31%) and civilians (64%) (52% female, 47% male). Subjects were grouped into four categories: current MDD (as determined by the SCID-4), current PTSD (as determined by the CAPS-5), both MDD and PTSD, and neither MDD nor PTSD. Using a two-way ANOVA (MDD yes/no and PTSD yes/no), the effects of MDD and PTSD diagnoses on symptomatology related to reward system dysregulation (measured by items D5-D7 on the CAPS-5) were examined. We found that MDD and PTSD both have significant main effects ($p<.01$) on symptoms related to reward-system dysfunction (D5-D7). Additionally a significant interaction between MDD and PTSD was found ($F(1,1)=6.105$, $p=.015$) when looking at feelings of social detachment (D6). This suggests that levels of social detachment are high in PTSD patients even when they are not depressed. Results support the hypothesis that the degree to which PTSD-sufferers experience reward system dysfunction cannot be solely explained by a comorbidity with MDD or subthreshold MDD.

Poster #19

The Relationship between Anxiety Sensitivity and Pubertal Status in Children and Adolescents with Social Anxiety

M. Falcone, E.R. Lebowitz, W.K. Silverman, C.E. Marin

Introduction: While no significant differences between boys and girls in their levels of anxiety symptoms, sex differences begin to emerge during adolescence with adolescent girls showing higher levels than adolescent boys. Adolescent girls also are vulnerable to elevated social anxiety symptoms. Higher anxiety sensitivity, which refers to individuals' beliefs that anxious physical symptoms may result in negative physiological, psychological, and social consequences, also has been found to be significantly associated with elevated social anxiety symptoms. The associations between pubertal status (rather than chronological age), anxiety sensitivity, and social anxiety have not been examined as far as we know in a clinical sample of anxiety disordered youth. Examining this issue is an important initial step to advance understanding of the divergence that emerges in youth anxiety, particularly social anxiety, in males and females. We hypothesized that early pubertal development will be associated with higher levels of anxiety sensitivity and higher levels of social anxiety symptoms in adolescent girls than boys. Methods: Participants were 230 youth (ages 6-14 years) who were evaluated at an anxiety disorders specialty research clinic. Youth and their parents signed assent and consent, respectively. Participants completed questionnaires assessing anxiety sensitivity, social anxiety, and pubertal status. Participants also participated in a semi-structured diagnostic interview at the initial evaluation to determine diagnostic status. All youth met criteria for an anxiety disorders diagnosis. Results: Preliminary analyses show that anxiety sensitivity was significantly and positively correlated with pubertal status; social anxiety symptoms were also significantly correlated with pubertal status. Conclusion: The results will be discussed with respect to understanding sex differences in males and females social anxiety levels and the role of anxiety sensitivity and pubertal status in advancing this understanding.

Poster #21

Emotion Dysregulation as a Moderator of Disability Stigma and Depression and Anxiety in Adults with Disabilities

R. Manning III, K. Wang

Despite making up 25% of the United States population, people with disabilities continue to face pervasive stigma. Little research, however, has examined how individual differences in stress coping, such as emotion

dysregulation (i.e., difficulties in understanding and modulating one's negative emotions), might moderate the association between stigma and mental health for this population. The present research considered the role of emotion dysregulation as a potential moderator of the association with stigma and depression and anxiety symptoms, while controlling for demographic and disability characteristics known to influence mental health. Data were collected from a larger study examining the psychosocial impact of COVID-19 on adults with disabilities in the United States. A total of 429 participants who self-identified as having a disability completed self-report measures on demographic and disability characteristics, disability-related stigma, depressive symptoms, anxiety symptoms, and emotion dysregulation. Moderation analyses revealed that perceived disability-related stigma was positively associated with higher levels of depressive and anxiety symptoms, but only for those individuals with higher levels of emotion dysregulation. These findings highlight the role of stigma as an important risk factor for depression and anxiety among people with disabilities, while simultaneously underscoring the potential utility of addressing emotion dysregulation in stigma coping interventions geared towards this population. Acknowledgements: Dr. Katie Wang and the Yale School of Public Health COVID-19 Rapid Response Pilot Gift.

Poster #23

Single-cell RNAseq of aging lungs from Nlrp3-/- mice reveals genotype- and celltype-specific effects of aging on the transcriptomes of several myeloid-derived cells

C. Cosme Jr., T.S. Adams, J.C. Schupp, J.E. McDonough, F. Ahangari, G. DeIuliis, N. Omote, V.D. Dixit, N. Kaminski

'Inflammaging,' an age-related phenomenon characterized by increased basal levels of proinflammatory molecules in circulation, has been suggested to contribute to the age-related decline in lung function and increased susceptibility to infection and disease. Mice lacking NLRP3, a pattern recognition receptor of the NLRP3 inflammasome, show reduced signs of 'inflammaging' and are a relevant model for studies on healthy aging. Here we report the preliminary results of a pilot single-cell RNA sequencing experiment of lungs from WT control and Nlrp3-/- mice of 2 months and 24 months old. From our single-cell RNA sequencing dataset of 41,878 cells from 7 subjects, we identified twenty-eight unique cellular types in lungs. Differential gene expression testing revealed noticeable genotype-specific differences in mRNA expression related to aging in the alveolar macrophages, interstitial macrophages and classical monocytes. We observed a reduction with age in Nlrp3-/- samples in the expression of several pro-inflammatory molecules related to the 'inflammaging' phenotype that include Tnf, Cxcl2, Chil3, Ccl3, and Ccl4. Interestingly, expression of Il1b increased noticeably with age in the macrophages and classical monocytes in the Nlrp3-/- samples. In addition to 'inflammaging,' the most pronounced genotype-specific differences were observable in old Nlrp3-/- interstitial macrophages which showed increases with age in expression of genes related to MHCII-mediated antigen presentation (Cd72, Cd74, various MHCII complex genes), proteasome subunits (Psma1, Psme1, Psme2, Psmb8), and anti-oxidation (Prdx5, Atox1). In summary, Nlrp3-/- knockout mice show distinct age-related transcriptomic changes in several myeloid-derived cell types of the lungs that highly express the NLRP3 inflammasome and control inflammaging. Additionally, several inflammation-related results agree with the general finding of reduced 'inflammaging' in aged Nlrp3-/- mice. These results suggest genotype-specific differences in the aging innate immune cell transcriptomes that may contribute to both the lung aging and global aging phenotypes. I would like to thank Dr. Kaminski for his guidance throughout this project and for his continued mentorship. Additionally, I would like to express special thanks to Jonas for processing the samples and generating the dataset as well as to Taylor and John for their technical guidance during analysis. Finally, I would like to thank

Dr. Dixit and his group for providing the mouse samples and their unique immunological insight during discussions on our results.

Session 2 Poster Program Abstracts

Poster #2

Micro-RNA-128 regulates the transdifferentiation of endothelial cells into blood stem cells

G. Baldissera, J.J. Ghersi, J. Hintzen, S. Nicoli

N/A

Poster #4

Uncovering the role of KIF12 in the pathogenesis of cholestasis

N. Dashti-Gibson, S. Lee, S. Vilarinho

We and others have identified that rare homozygous damaging mutations in KIF12, which encodes kinesin family member 12, cause high gamma-glutamyltransferase cholestasis. While KIF12 has been investigated in the contexts of diabetes and polycystic kidney disease, its role in biliary cell development and function remains largely unknown. Notably, in the human liver, we have found that KIF12 is expressed exclusively in biliary epithelial cells, also known as cholangiocytes. Since KIF12 is a microtubule-associated motor protein, we hypothesize that loss of KIF12 may disrupt the formation of cilia, microtubule-based structures that help cholangiocytes respond to changes in bile flow and composition. For this purpose, we have generated human induced pluripotent stem cell (iPSC) lines with a patient-specific mutation using a CRISPR/Cas9 gene editing approach. We have successfully differentiated wild-type, KIF12-heterozygous, and KIF12-homozygous iPSC lines through definitive endoderm, hepatic endoderm, hepatoblast, and cholangiocyte stages, as confirmed by gene expression analysis. We have found that independent of their genotype, all three iPSC lines successfully differentiate into cholangiocytes, with expression of CK19 and CK7 at the mRNA and protein levels. We have also generated iPSC-derived biliary organoids to further evaluate cilia formation and biliary function in the presence and absence of KIF12. In summary, we have newly generated both 2D and 3D iPSC-derived biliary models to study human KIF12 deficiency, the pathogenesis of which remains elusive. By understanding the mechanism by which mutations in KIF12 lead to severe cholestasis, we aim to identify novel therapeutic targets for this rare disease and possibly other cholestatic liver diseases and gain new insight into liver biology. Our laboratory is supported by Doris Duke Charitable Foundation Grant #2019081.

Poster #6

Hippo/Yap signaling limits endogenous neuroregeneration in human retina

S. Donepudi, R.M. Dhodhapkar, E. Calapkulu, Y. Xing, L. Zhang, M. Menon, A. Dong, B.P. Hafler

Normally, your eye is secreting fluid, which is important in maintaining shape of the eye. Proper drainage accordingly, is a sign of good eye health. In cases where the drainage channel trabecular meshwork, is blocked,

glaucoma can result. In POAG, this drainage is blocked, due to stiffening of the drainage canals for this fluid. As a result, pressure builds up, causing retinal ganglion cells die, resulting in vision loss. Current treatments are invasive, have severe side effects, nor cure glaucoma-- but they will prevent it from getting worse; furthermore, it doesn't solve the RGC death problem. Activating the retinal endogenous regeneration and repair is the most direct treatment for RGC loss. In certain amphibians, i.e. zebrafish, the retinal Müller cells have regenerative capability, and are able to fully restore the retinal tissue, even after severe induced retinal damage. In humans, this is greatly inhibited. Previous studies have suggested that a few pathways may play key roles in neuroregeneration including the embryogenic Hippo/Yap pathway. In mice, inhibition or bypass of the pathway, has allowed transgenic mice to mount a proliferative response to damage from intravitreal NMDA (N-methyl D-aspartate) injection. Recently, A.J. Hudspeth's lab has shown that the use of The Rockefeller University Lats Kinase Inhibitor (TRULI), may promote Yap-dependent proliferation in postmitotic mammalian tissues. Other lab member's ongoing work identified that the Hippo/Yap pathway is downregulated in zebrafish, and upregulated in the human eye via pseudotime analysis. Ongoing labwork involves using this to see whether or not inhibition prevents Yap phosphorylation and induces proliferation of supporting cells in the human eye. Planned experiments include establishing the timecourse in which the Muller glia start proliferating, and maybe establish the optimal therapeutic window to treat glaucoma. Additionally, any downstream/upstream genes of the Hippo/Yap pathway can be further tested to identify particular targets for therapeutic intervention. We would like to respectfully acknowledge the eye donors and their families, without whose sacrifice this research would not be possible.

Poster #8

Examining the Spatial and Temporal Elements of the Interferon Gamma Signaling Pathway

B. Horowitch

Interferon gamma (IFNg) is a cytokine known to have both anti- and pro-tumorigenic effects. Primarily produced by T cells and Natural Killer cells, it increases immune activation and promotes tumor clearance by upregulating class I MHC, which presents endogenous antigens, on tumor cells. Counterintuitively, chronic IFNg stimulation has been found to foster resistance to immunotherapy, and IFNg is known to upregulate inhibitory molecules on the surfaces of tumor cells. This indicates that the types of cells producing and responding to IFNg, as well as the timing of IFNg signaling, are crucial for evaluating its role in tumor immunology, but these two aspects of the pathway have not been well-characterized. To clarify the spatial elements of IFNg signaling and determine which cells make and which sense IFNg, we are combining in situ hybridization and quantitative immunofluorescence to stain for IFNg mRNA, phosphoSTAT1 (pSTAT1), and programmed death-ligand 1 (PD-L1) in tumor microarrays. Cells positive for IFNg mRNA generate the cytokine while those positive for pSTAT1, a transcription factor downstream of IFNg, and PD-L1, an inhibitory ligand upregulated by IFNg, respond to it. By staining clinically-annotated samples, we will establish how the location of IFNg signaling in the tumor microenvironment correlates with patient outcomes. To investigate how the timing of IFNg signaling promotes or inhibits tumor clearance, we have constructed an IFNg fluorescent reporter in A375 melanoma cells. We will conduct genome-wide CRISPR screens to identify gene knockouts that increase the acute, but not chronic, response to IFNg stimulation then validate and study these targets. We have optimized the staining protocol in T cells and tumor cell lines and constructed the IFNg fluorescent reporter. When we stain the tumor samples and conduct the CRISPR screens, we hope to develop a better understanding of the IFNg pathway, which may help to improve cancer immunotherapies. Acknowledgements: Anna Word Sarah Rodwin Kathryn Clulo Therese

Cordero Dumit Eejung Kim, MD, PhD Sandra Martinez-Mortilla, PhD David Rimm, MD, PhD Jeffrey Ishizuka, MD, DPhil.

Poster #10

An integrative single-cell framework to reveal insights into the tumor microenvironment and characterize the responses of patients to therapeutic perturbations

F Ouerghi

The recent increasing availability of the varying single-cell technologies has increased our knowledge of the composition of the tumor microenvironment in various cancers, and yet, there is still a lack of application of single-cell approaches to interrogate the mechanisms of immune checkpoint blockade resistance in Merkel cell carcinoma and head and neck cancers. Here, we developed a single-cell framework that studies the dynamic response to perturbation using patient samples enabling the comparison of multiple therapeutic perturbations in a single cell experiment. This approach, called PERCEPT (PERturb-seq of Coculture from Ex vivo Patient Tumors), enables the simultaneous measurement of transcriptional, oligonucleotide-tagged protein and T cell repertoire expression in multiplexed patient samples co-cultured using different stimulations for a period of 48 hours. We put in place a single computational framework that integrates the demultiplexing of cell hashing results, the detailed analyses of the results of single-cell RNA-sequencing and surface protein measurements, the profiling of T cell receptor receptoires, the reverse engineering of gene regulatory networks, the integration of the patient samples to minimize batch effects, the use of diffusion maps, cosine similarity and Pearson's correlation indices, and the incorporation of surprisal and critical point analyses. The consolidation of these techniques into a single pipeline facilitates the interrogation of the differences in the responses of patients to the same therapeutic stimulations by looking at the differences in trajectories cell subtypes undertake in response to different stimulations. Applying these tools to freshly resected surgical samples, we are able to perform a multiplexed comparison of a single cell sample to systematically understand the mechanisms in which the stimulations are driving the immune reactions, uncover new targets to potentially overcome the resistance to immune checkpoint blockade therapy, and dissect the role of hypoxia in inducing immune dysfunction in these tumor immune co-cultures. I would like to express my absolute gratitude to Dr. Jeffrey Ishizuka who has provided constant mentorship and guidance in exploring this research topic from a computational perspective. I would also like to thank Dr. Jessica Wei for her mentorship and the leadership of the wet lab aspect of this project. I also extend my gratitude to all the members of the Ishizuka lab who have provided ample support and tools to undertake this research.

Poster #12

The durability of immunity against reinfection by SARS-CoV-2: a comparative evolutionary study

H. Hassler, J.P. Townsend, Z. Wang, S. Miura, J. Singh, S. Kumar, N.H. Ruddle, A.P. Galvani, A. Dornburg

Among the most consequential unknowns of the devastating COVID-19 pandemic are the durability of immunity and time to likely reinfection. There is limited direct data on SARS-CoV-2 long-term immune responses and reinfection. However, the durability of immunity among evolutionarily close coronavirus relatives of SARS-CoV-2 has been assayed, making it possible to estimate its duration of immunity by a comparative evolutionary analysis of related viruses SARS-CoV-1, HCoV-MERS, HCoV-229E, HCoV-OC43, and HCoV-NL63. We integrated comparative phylogenetic approaches with analysis of Nucleocapsid, Spike, and whole-virus lysate immunoglobulin G (IgG) antibody optical density levels, in conjunction with reinfection data on human

seasonal coronaviruses. We estimated the expected decline in antibody levels over time, the probability of reinfection based on antibody level, and the anticipated time to reinfection. Reinfection by SARS-CoV-2 under endemic conditions would likely occur between 3 and 63 months, with a median of 16 months. This protection is of less than half the duration revealed for the seasonal coronaviruses circulating among humans. The time frame for reinfection is fundamental to myriad aspects of public health decision-making. As the pandemic continues, reinfection is likely to become increasingly common. Maintaining public health measures that curb transmission—including among individuals who were previously infected by SARS-CoV-2—coupled with persistent efforts to accelerate vaccination are critical to prevention of COVID-19 morbidity and mortality. This research was funded by the National Science Foundation of the United States of America RAPID 2031204 to JPT and AD, RAPID 2034228 to SK and SM, and NSF Expeditions CCF 1918784 to JPT and to APG.

Poster #14

Insights into Scrub Typhus: Biochemical Characterization and Structure of a Guanine Nucleotide Exchange Factor Encoded by an Effector Protein from Orientia tsutsugamushi

A. Blaise, J.M. Berk, C. Lim, A.J. Koleske, M. Hochstrasser, and Y. Xiong

Scrub typhus, a tropical disease caused by intracellular bacterium Orientia tsutsugamushi, affects about 1 million people each year. Symptoms range from nausea to organ failure and can become fatal if not treated with antibiotics. Through computational screening, we have identified and characterized a protein expressed during O. tsutsugamushi infection. HeLa cell lysate pulldowns of this protein paired with mass spectrometry identified RhoGTPases Rac1 and Cdc42 as candidate binding partners. Intracellular pathogens often secrete guanine nucleotide exchange factor (GEF) domains to disrupt cytoskeletal signaling and host actin networks to facilitate cellular invasion. These findings led us to hypothesize the presence of a potential GEF domain in the protein. This study focused on measuring and understanding the putative GEF activity of this protein. We find that this protein can catalyze the exchange of BODIPY-FL-GDP for GTP on both Rac1 and Cdc42, causing a decrease in fluorescence in a time- and GEF concentration-dependent manner. This GEF activity was mapped to a central part of the protein (OtGEF domain). To further understand the interaction of the OtGEF domain with host GTPases, we solved a 3.0 Å crystal structure of the apo OtGEF domain, as well as a 1.7 Å resolution structure of the OtGEF:Rac1 complex. The crystal structure revealed that OtGEF has a novel, V-shaped fold not seen in any other bacterial effector GEF to date. Furthermore, the complex structure revealed that OtGEF coordinates an extensive network of ordered water molecules controlled by a glutamate residue that promotes nucleotide exchange. Mutation of this catalytic glutamate reduced GDP dissociation to background levels, confirming its importance for GEF activity. Our studies demonstrate that the O. tsutsugamushi putative effector protein contains a GEF domain that acts on the GTPases Rac1 and Cdc42, and suggests a critical role for this protein in manipulating host actin dynamics during infection. Funded by NIH Grant NIH R01 MH115939-01

Poster #16

Investigating the Impact of Maternal Immune Response on Microglia During the Fetal Neural Development

H. Lee, A. Lu-Culligan, A. Iwasaki

N/A

Poster #18

Hyperspectral Imaging in Systemic Sclerosis-Raynaud Phenomenon

A. Williams, N. Page, S. Teaw, A. Gupta, F.P. Wilson, B.J. Sumpio, B.E. Sumpio, M. Hinchcliff

Introduction: Raynaud phenomenon (RP), a microcirculatory, vasospastic disorder, may be primary or secondary to an autoimmune disease [e.g., an early indicator of systemic sclerosis (SSc)]. RP clinical trials have been hampered by the lack of robust, feasible, and quantitative methodology for assessing change over time. Hyperspectral imaging (HSI) noninvasively measures oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) concentrations, and oxygen saturation (O2 sat) in the skin, depicted as an oxygenation heatmap. We aim to explore the potential role of HSI in quantifying SSc-RP hand microcirculation. Methods: The Yale University Human Investigation Committee approved this study (HIC# 2000026608) of Yale SSc patients and healthy controls (HCs). Bilateral palmar HSI (HyperMed™, Waltham, MA) was obtained in a temperature-controlled room. OxyHb, deoxyHb and O2 sat values were calculated using a region of interest measuring 78 mm2 on the fingertips and palm. Subjects then submerged their hands in 15°C water for 1 minute, and HSI was repeated at 0, 10, and 20 minutes. Statistical analysis was performed using the mixed-effects model. Results: Baseline oxyHb, deoxyHb and O2 sat maps did not significantly differ between SSc and HC participants. After cold provocation, images from baseline to 0 min revealed significant reductions in mean slopes of oxyHb and O2 sat for SSc (-0.71, -0.33) compared to HC (-0.22, -0.11; p=0.005, 0.014, respectively). From 0 to 20 min, SSc subjects had more change in oxyHb (p=0.001), deoxyHb (p=0.003), and O2 sat (p<0.001). Conclusion: HSI is a feasible approach for microcirculation measurement in the hands of SSc patients. SSc subjects had a greater decline in oxyHb and O2 sat values from baseline to time 0 compared to HC subjects. These data suggest that HSI technology to assess RP vascular dysfunction is a potential quantitative measure for SSc-RP severity and activity in clinical trials. The Yale University Human Investigation Committee approved this study (HIC# 2000026608).

Poster #20

Analyzing the Cellular Composition of Tertiary Lymphoid Structures in Mouse Models of Lung Adenocarcinoma

D. Mariuzza, K.A. Connolly, C. Cui, N.S. Joshi

Immunotherapies have revolutionized the cancer treatment landscape, but many patients still do not respond to these therapies. In non-small cell lung cancer, only ~20% of patients treated with immune checkpoint blockade achieve durable responses. Identifying markers predictive of clinical response is vital to overcoming this challenge. The presence of tertiary lymphoid structures (TLS), organized structures of immune cells that develop at the tumor site, often correlate with favorable patient responses. Despite this, the mechanism by which they impact the anti-tumor immune response remains insufficiently understood. Moreover, variations in the cellular composition of TLS present further barriers to determining their utility as prognostic tools. Previously, we demonstrated that TLS formation depends on the presence of tumor antigen. We hypothesize that the cellular composition of TLS is determined by the types of tumor antigens expressed, resulting in differential therapeutic potential. Specifically, we anticipate that the presence of tumor-associated B cell antigens will promote increased B cell populations and more mature structural elements, such as germinal centers within TLS. To test this, our lab developed two mouse models of lung adenocarcinoma, KP-NINJA and KP-HELLO, in which tumors express identical T cell antigens alone or in combination with a B cell antigen, respectively. Immunofluorescence imaging was conducted to assess TLS in the KP-NINJA model. We found that 31/60 tumors contained at least one associated TLS, defined by a region of >20 T and/or B cells. Of these, all contained T cells, and 26/31 also contained B cells. We will further characterize the phenotypes of these cell populations and compare these TLS to those in the T and B cell antigen-expressing KP-HELLO model.

Importantly, future studies will test the resulting impacts on tumor growth and therapy responsiveness. Utilizing these models will ultimately provide a clearer view of the clinical prognostic potential of TLS.
Acknowledgments: American Lung Association.

Poster #22

A hairpin to duplex conversion underlies RNA foci phase transitions

E. Acks, C.M. Davis

N/A

Poster #24

RNase A Folding in Response to Hypoxia

B. Ramsey, C. Davis

Hypoxic conditions, decreased availability of oxygen, disrupt glycosylation and disulfide bonding, processes that are necessary for correct protein folding and secretion. In particular, oxygen is critical as a terminal electron acceptor for disulfide bond formation in oxidative protein folding. Here we measure the stability and folding of ribonuclease A (RNase A) Förster resonance energy transfer (FRET) constructs in vitro and in eukaryotic cells. There are eight cysteine residues in RNase A that form four disulfide bonds in the native protein. Despite being a highly-used test protein for folding studies, RNase A still has a number of properties that are unresolved, including the full characterization of its oxidative folding pathway in cells. Our hypothesis is that folding of RNase A is oxygen dependent. First, we design a FRET reporter on protein folding in vitro that minimally perturbs RNase A's stability and enzymatic activity. Then, we compare the folding of RNase A in cells under normal conditions and oxidative stress. cobalt chloride simulates hypoxic conditions. This work may inform therapeutic interventions to stall or promote protein folding in the context of cancer, diabetes, or neurodegenerative disease.