

## 2023 Yale Postdoctoral Association Symposium

### Poster Session Details

Location: Lobby of OC Marsh Hall

#### Poster Session 1

Time: 11:00am-12:00pm

<b>Presenter (Submission ID)</b>	<b>Title</b>
Jyot Antani (5512306)	<i>High-throughput screening of drug candidates for phage therapy</i>
Faranak Ebrahimian Sadabad (5689550)	<i>The utility of Tau PET imaging in Frontotemporal dementia: A meta-analysis</i>
Sabrina Ghadaouia (5647984)	<i>The role of nuclear F-actin in oocyte genomic integrity</i>
Justin Lee (5642786)	<i>Examining Proton-Coupled Electron Transfer and Oxygen-Atom Transfer in Aqueous Iridium Oxide Nanocrystals</i>
Anna Lo (5789789)	<i>Mechanistic studies of KL-50, a novel imidazotetrazine for the treatment of MGMT-/MMR- gliomas and glioblastoma.</i>
Jacqueline Mann (5792081)	<i>Advocacy strategies driving positive change for Yale postdoctoral researchers</i>
Natasha Pinto Medici (5768708)	<i>Altered RNA splicing causes pancreatic cancer and exposes a therapeutic vulnerability</i>
Md Afjalus Siraj (5773903)	<i>Phosphorylation of ERK is controlled during its pre-mRNA splicing in pancreatic cancer</i>
Juan Velasco (5651579)	<i>Long-Term Outcomes of the Ross Procedure Versus Bioprosthetic Versus Mechanical Aortic Valve Replacement: A Network Meta-Analysis</i>
Brionna Davis-Reyes (N/A – Display Only)	<i>Postdoc Belonging in Academia: The Powerful Impact of BIPOC founded Organizations on Postdoc Success</i>
Kate Nyhan (N/A – Display Only)	<i>Postdocs and all research staff are supported and welcomed by Cushing/Whitney Medical Library</i>
Ziv Ben-Zion (5747234)	<i>Are We There Yet? Evaluating the Evidence for Neuroimaging-Based Biotypes of Psychiatric Vulnerability in the Acute Aftermath of Trauma</i>
Todd Douglas (5655385)	<i>A maladaptive stress response promotes cell death during nutrient starvation</i>
Snimarjot Kaur (5777331)	<i>Blueprint for Community Emergency Department Pediatric Simulation Curriculum: From PECC to Practice</i>
José Martínez-Magaña (5785207)	<i>Genome-wide association study of alcohol consumption identifies novel loci in Latin American populations: Findings from the Latin American Genomics Consortium</i>

**Poster Session 2**

Time: 12:15pm-1:15pm

<b>Presenter (Submission ID)</b>	<b>Title</b>
Debanjan Bhattacharjee (5704585)	<i>A drug's most potent target is not necessarily the source of its anti-cancer activity</i>
Jun He (5704665)	<i>Sex differences in the pleiotropy of hearing problems with imaging-derived phenotypes: a brain-wide imaging study</i>
Waleed Ibrahim (5567956)	<i>Seasonal variation in 5-HT<sub>1B</sub> receptor availability in healthy individuals</i>
Magdalena Mijas (5794461)	<i>The interplay of testosterone and cortisol response to acute stress in the context of minority stress</i>
Aditi Naskar (5654473)	<i>Colocalization of phosphorylated TDP-43 with organellar proteins in TDP-43 positive AD patients</i>
Alaz Özcan (5771736)	<i>Chemokine producers reveal tissue-specific neutrophil niches.</i>
Barbora Salovska (5571720)	<i>Phosphoproteomic analysis of metformin signaling in colorectal cancer cells elucidates mechanism of action and potential therapeutic opportunities.</i>
Juan Velasco (5651606)	<i>Outcomes of the V-shaped noncoronary sinus remodeling technique for ascending aortic aneurysm and aortic root ectasia</i>
Manjula Ramu (5651998)	<i>Engineering of the high affinity chemokine CXCL13 to screen CXCR5 antagonists to treat cancer and autoimmune diseases</i>
Lisa Baik (5662371)	<i>The taste of humans and nectar: gustation in the Asian tiger mosquito</i>
Divyani Goyal (5355627)	<i>Quantification of Cardiac Amyloidosis from Tc-99m Pyrophosphate Planar Images Using a New Semi-automated Approach: Intra- and Inter-observability assessments in Patients</i>
Rajesh Kar (5704726)	<i>Ssu72 mediated Pol II pausing at exon 2 is essential for co-transcriptional processing</i>
Krishna Mudumbi (5784371)	<i>Are the kinetics of EGFR clustering and ERK signaling connected?</i>
Zhenlan Wang (5781952)	<i>Teachers' intervention of conflicts between children: A cross-cultural comparison between Chinese and American early childhood educators</i>

## **High-throughput screening of drug candidates for phage therapy**

**Jyot Antani**, Timothy Ward, Isabella Graf, Thierry Emonet, Paul E Turner

Bacteriophages (phages) are emerging as promising therapeutics to treat multi-drug-resistant bacterial infections, a major biomedical challenge. An important determinant of phage therapy efficacy is the attachment (adsorption) efficacy of phages to the host surface. The classical understanding of phage adsorption is derived from flasks and plate-based assays, which provide ensemble estimates of the adsorption rate. Characterizing the stochastic dynamics of phage-host interactions requires single cell and single phage measurements. We developed a fluorescence microscopy-based assay to quantify the attachment of individual phages to cells. We employed particle tracking algorithms to obtain single phage trajectories from videos recorded with high spatiotemporal resolution. Tracking phage T4 particles near host *Escherichia coli* surfaces revealed that phages reversibly bind to and unbind from host surfaces. From thousands of trajectories, we obtained histograms of the dwell time, the time that a phage spends near bacterial cells, likely exploring the surface. These histograms do not follow exponential distributions as predicted by the existing theory of phage attachment. We propose an updated model of the biophysics of phage adsorption. Simulating this model produces the outcomes of our single-phage microscopy assays as well as the classical adsorption assays. Next, we compared the dwell time distributions for phages attaching to wildtype host cells and cells of multiple strains carrying mutated phage-receptors. As expected, the dwell times of phages near the mutant cell surfaces are lower. Comparisons of the classical adsorption rate to the dwell time distributions revealed a monotonic relationship. These results establish a framework for quantifying the dynamics and stochasticity of the interactions between viruses and the host surface. Our assay is generalizable as we use a nonspecific dye for fluorescence-labeling. This microscopy assay can be used for rapid, high-throughput screening of phage candidates for their adsorption efficacy, thus providing a powerful approach for emergency phage therapy.

## **The utility of Tau PET imaging in Frontotemporal dementia: A meta-analysis**

**Faranak Ebrahimian Sadabad**, Dongning Su, Seyed Faraz Nejati, Arman Fesharaki-Zadeh, Yanghong Yang, David Matuskey

**Background:** (PET) tracers bind to the paired helical filaments of tau in aging and (AD) but its utility in detecting tau aggregates in (FTD) is less certain.

**Objective:** To provide an aggregated quantitative analysis of the value added by tau (PET) imaging in FTD patients compared to healthy participants.

**Method:** Systematic search was performed in PubMed, Web of Science, Embase, and Scopus until June 2022. 151 FTD patients and 257 healthy controls over 9 studies were included. Data were abstracted by four observers and assessed for publication bias. The (SUVR) in FTD patients and healthy controls with tau PET imaging were compared in the frontal, temporal, and parietal cortices.

**Result:** FTD patients showed an increase in SUVR in frontal, temporal, and parietal lobes. The sensitivity analysis showed similar pooled estimates in these four lobes, indicating our meta-analysis results are relatively reliable.

**Conclusion:** This is the first meta-analysis of tau PET in FTD and we confirm higher uptake value in FTD in the frontal, temporal, and parietal regions compared to controls and provide evidence it may be useful in determining the variant of FTD.

## **The role of nuclear F-actin in oocyte genomic integrity**

**Sabrina Ghadaouia**, Federica Giannini, Kathleen Scheffler, Binyam Mogessie

Efficient DNA repair is critical for animal development. Mammalian oocytes are remarkably susceptible to DNA damage and accumulate DNA lesions with advancing maternal age, a phenomenon whose molecular basis remain unknown. The actin cytoskeleton has recently emerged as a protector of oocytes against chromosomal abnormalities. We recently discovered that mammalian oocyte nuclei contain prominent actin filaments that are significantly reduced in reproductively older females. Our new preliminary data show that oocyte genome damage causes excessive assembly and bundling of nuclear F-actin cables. Based on these observations, we hypothesize that nuclear F-actin structures promote DNA repair and that their disruption underlies reproductive aging-related accumulation of DNA damage in oocytes. To test this exciting hypothesis, we are using a combination of advanced microscopy, localized nuclear F-actin degradation, and DNA damage induction assays. We are inducing DNA damages using X-Ray irradiation or pharmacological treatments, which provide fine control over the amount of DNA damages regenerated. The specific degradation of F-actin is achieved using TRIM-away, an antibody-based technique that uses the ubiquitin ligase TRIM21 to specifically target proteins to the proteasome. Combining these techniques with high-resolution live and immunofluorescence microscopy assays, we can analyze DNA damage response and repair in the presence or absence of nuclear F-actin. These integrated approaches could reveal new actin-based mechanisms of genomic integrity and provide new insights into the causes of reproductive age-related female infertility.

## **Examining Proton-Coupled Electron Transfer and Oxygen-Atom Transfer in Aqueous Iridium Oxide Nanocrystals**

**Justin Lee**, James M. Mayer

Reactivity at the metal-oxide/water interface is increasingly recognized as proton-coupled electron transfer (PCET), rather than the traditional view based on just electron transfer and parameters such as band energies. For instance, iridium oxide (IrOx) bulk material or nanocrystals (NCs), which are some of the most efficient water oxidation electrocatalysts, have been demonstrated to undergo PCET processes by others using various electrochemical methods. Our approach is to quantify the thermodynamic properties of these metal-oxide NCs using stoichiometric chemical reactions. In this poster, we will present the synthesis and physical characterization of aqueous colloidal IrOx NCs, and how they change in acidic versus basic pHs. The IrOx NCs are treated with chemical reactants that promote e-transfer or the coupling of  $n$  H<sup>+</sup>/ $n$  e<sup>-</sup> movements, which are monitored spectroscopically. This allows for the bracketing the PCET thermochemistry, in other words the [IrOx]-H bond dissociation free energy. We find that roughly all IrIV/III centers are redox active, consistent with prior electrochemical and catalytic results. We will also demonstrate the utility of IrOx NCs to perform oxygen-atom transfer reactions to phosphine and thioether reagents, and preliminary findings suggest the rate of these reactions are dependent on the average oxidation states of the NCs. These results can provide an increased understanding of reactions occurring at the metal-oxide/solution interface in the design of future catalysts.

## **Mechanistic studies of KL-50, a novel imidazotetrazine for the treatment of MGMT–/MMR– gliomas and glioblastoma**

Eric D. Huseman, **Anna Lo**, Olga Fedorova, Kingson Lin, Susan E. Gueble, Ranjini K. Sundaram, Anna M. Pyle, Ranjit S. Bindra, and Seth B. Herzon

The DNA damage repair protein O6-methylguanine methyl transferase (MGMT) reverses O6-alkylguanine lesions via SN2 transfer of the lesion to an active site cysteine. MGMT is ubiquitously expressed in healthy tissue but is silenced by promoter hypermethylation in approximately half of glioblastomas and up to 75% of lower grade gliomas. As such, patients with MGMT deficient (MGMT–) tumors benefit from DNA alkylating agents such as temozolomide (TMZ), an imidazotetrazine that deposits methyl lesions at O6-guanine. However, as the cytotoxicity of TMZ requires an intact DNA mismatch repair (MMR) pathway, acquired resistance to TMZ via MMR silencing negates the efficacy of the drug. We recently reported the synthesis, discovery, and in vivo evaluation of KL-50, a TMZ analogue that overcomes this resistance mechanism while maintaining high selectivity for MGMT– cells in vitro and in vivo. Herein, we provide mechanistic in vitro and in vivo studies to show that KL-50 generates an O6-(2-fluoroethyl)guanine (O6FetG) lesion that is rapidly repaired in MGMT+ cells. However, in MGMT– cells, the initial O6FetG lesion slowly cyclizes to N1,O6-ethanoguanine (N1O6EtG) which further reacts with the base-paired cytidine residue to give a G(N1)-ethyl-C(N3) interstrand crosslink (ICL), causing cell death independent of MMR status. These findings are consistent with our mechanistic model and suggest that strategies that exploit the relative rates of DNA damage and repair may extend to other tumor types harboring specific DNA repair defects.

## **Advocacy strategies driving positive change for Yale postdoctoral researchers**

Nicole J. Lake, **Jacqueline E. Mann**, Anna York, Azmi Ahmad, Anderson Brito, Krishna C. Mudumbi

A key part of the Yale Postdoctoral Association's (YPA) mission is to advocate for the 1200+ postdocs they serve, by representing their interests to the Schools and Offices of Yale University. The COVID-19 pandemic exposed unmet needs within the postdoc community at Yale. These issues were addressed by new avenues through which postdocs could directly interface with faculty and administration. The positive outcomes achieved from these conversations during the pandemic led to the establishment of the Trainee Accommodations Committee, which provides a monthly forum for the YPA to raise issues emerging for the postdoc community with university leadership. Additional monthly meetings between the YPA and the Office of Postdoctoral Affairs (OPA) augment support with advocacy-related topics, and enable collaborations on initiatives to support postdocs across campus. Through these meetings and related strategies, the YPA has successfully advocated for policy changes to improve the postdoctoral experience. This includes achieving pay equity by ensuring salary increases are not contingent on reappointment date, a prior policy which produced inequities among postdocs of the same year, and providing postdoctoral fellows with equal access to childcare and healthcare benefits, overcoming their historical categorization as benefits-ineligible employees. The latter was achieved via the establishment of a fund to subsidize the increased health insurance costs for postdoctoral fellows, making their costs equivalent to postdoctoral associates. These experiences also emphasized the key role that allyship of faculty and staff can play in advocacy success. Ongoing work includes advocating for an ombuds office at Yale to support postdocs navigating conflict resolution, and a survey in collaboration with OPA to identify unmet needs in the

postdoc community. This enables data-driven negotiations with university leadership to support our efforts to address issues affecting postdocs.

### **Altered RNA splicing causes pancreatic cancer and exposes a therapeutic vulnerability**

**Natasha Pinto Medici**, Diana Martinez-Saucedo, Danny Lee, Li-Ting Ku, Robert Tseng, Deanne Yugawa, Sumedha Chowdhury, Jeffrey Townsend, Vincent Cannataro, Christine Iacobuzio-Donahue, Omar Abdel-Wahab, Steven D. Leach and Luisa Escobar-Hoyos

Pancreatic cancer (PC) is the fourth leading cause of cancer deaths in the Western world. Many factors play a role in this phenomenon, including the difficulty in diagnosing this disease in its early stages and the fast development of therapy resistance. Thus, there is an unmet need to understand the pathogenesis of PC and how they develop resistance to the currently available chemotherapies. In our laboratory, we discovered that the pathway responsible for the creation of the wide array of proteins we have in our cells – a mechanism called RNA Splicing (RS) – is a hallmark of the most aggressive PC. We observed that in these cancer cells, this pathway is modified, leading to the generation of defective products that contribute to cancer formation and maintenance. We also observed that either targeting members of this pathway or the generated defective products were detrimental to the cancer cells, indicating that this could new therapeutic strategy. Therefore, we aim to answer two important questions: 1) Is defective RS driving PC pathogenesis? and 2) is defective RS contributing to resistance to therapy? To answer those questions, we used different PC models to recapitulate the features of human cancer. We observed a widespread RS defect in cancer cells, that occurs in the same manner in all models. We also detected that once certain defects are triggered, a diverse array of proteins is eliminated, including proteins that can combat tumor formation. Our next research steps are to use a therapy designed in our laboratory called Splicing-Hit Oligonucleotide Therapy (SHOT). This therapy will correct RS defects identified in cancer cells to restore their normal activity and consequently eliminate tumors. The data from these studies have the potential to accelerate the generation of novel personalized therapies for cancer treatment.

### **Phosphorylation of ERK is controlled during its pre-mRNA splicing in pancreatic cancer**

**Md Afjalus Siraj**, Yushan Zhang, Prabir Chakraborty, Robert Tseng, Li-Ting Ku, Grant Goda, Gilbert Giri, Shamik Das, Anindya Dey, Shailendra Kumar Dhar Dwived, Geeta Rao, Min Zhang, Da Yang, Md Nazir Hossen, Wei-Qun Ding, Kar-Ming Fung, Resham Bhattacharya, Daniel Dominguez, Luisa Escobar-Hoyos, Priyabrata Mukherjee

Many carcinomas increase the expression of RNA splicing factors, however, the functional consequences are largely unknown. Here, we demonstrate that SMNDC1, a poorly studied splicing factor, which we found to be upregulated in multiple carcinomas and associated with poor patient prognosis, promotes tumor growth and resistance to chemo and targeted therapies by promoting the retention of G-rich cassette exons, which otherwise would be excluded or retained at a lower rate after RNA splicing in normal cells. Across tumors, SMNDC1 promotes the inclusion of cassette exon 4 (E4) of MAPK3 (ERK1), which encodes the kinase-activating phosphorylation sites (Thr202/Tyr204). Forced exclusion of MAPK3-E4 using anti-sense oligos or CRISPR-directed mutations at E4 splice site inhibited exon retention, ERK1 phosphorylation, expression of ERK-target genes, and tumor establishment. Increased retention of E4 by SMNDC1 was identified as a mechanism to resist chemotherapeutic agents (Gemcitabine and 5FU), and small-molecule inhibitors against EGFR and MEK, all upstream of ERK. SMNDC1 recognizes an A/C-rich

element in the 3' intron of MAPK3-E4 promoting its retention through a sequence independent of the reported RNA-binding Tudor domain. These data support that cancer cells exploit a “splicing switch” to promote ERK kinase activity and resistance to therapies, independent of activating mutations in the RAS/RAF/MAPK pathway, and offer a druggable alternative to block oncogenic signaling and altered RNA splicing in cancer cells.

### **Long-Term Outcomes of the Ross Procedure Versus Bioprosthetic Versus Mechanical Aortic Valve Replacement: A Network Meta-Analysis**

**Juan J. Velasco**, Zachary G. Perez, Mohammad A. Zafar, John A. Elefteriades

The choice of the aortic valve substitute for young and middle-aged adults is still debated. This study aims to simultaneously compare the direct and indirect evidence of the clinical outcomes following Ross procedure, bioprosthetic aortic valve replacement (bAVR), and mechanical aortic valve replacement (mAVR). After a systematic literature search, randomized clinical trials and propensity score matched studies comparing any combination of Ross procedure, bAVR, and mAVR were included. Twenty-five studies with a pooled sample size of 110,023 patients (Ross procedure, n=1,691; mAVR, n=54,811; bAVR, n=53,521) met the eligibility criteria. A frequentist network meta-analysis and a random-effects pairwise meta-analysis were performed. Primary end points were in-hospital mortality and all-cause mortality at last follow-up. Secondary end points were stroke or transient ischemic attack, bleeding, endocarditis, and reoperation. All-cause mortality at last follow-up was significantly lower in patients with Ross procedure compared to those with mAVR (OR 0.59; 95%CI 0.41–0.80; P<0.01) and bAVR (OR 0.44; 95%CI 0.25–0.77; P<0.01). Stroke or transient ischemic attack was significantly lower in Ross procedure compared to mAVR (OR 0.31; 95%CI 0.12–0.80; P=0.02), whereas it was equivalent to bAVR. Compared to mAVR, Ross procedure was associated with a significantly lower risk of bleeding (OR 0.17; 95%CI 0.03–0.92; P=0.04). Regarding reoperation, it was significantly lower in Ross procedure compared to bAVR (OR 0.37; 95%CI 0.18–0.75; P<0.001), whereas no evidence of difference was found compared to mAVR. Endocarditis was equivalent in patients with Ross procedure compared to mAVR (OR 0.59; 95%CI 0.31–1.13; P=0.11) and bAVR (OR 0.39; 95%CI 0.13–1.14; P=0.08). No evidence of difference was found between the three approaches for in-hospital mortality. Our study shows that Ross procedure has a significantly lesser likelihood of long-term outcomes such as all-cause mortality at last follow-up, stroke or transient ischemic attack, bleeding, and reoperation compared to conventional aortic valve replacement.

### **Postdoc Belonging in Academia: The Powerful Impact of BIPOC founded Organizations on Postdoc Success**

**Brionna D. Davis-Reyes**, Christine Vazquez, Kristyn A. Carter, Michelle G. Thompson, and Walatta-Tseyon Mesquitta

Historically, academia has ignored and discounted not only the voices but the talent of scientists identifying as Black, Indigenous, and/or People of Color (BIPOC) and thus led to the overwhelmingly exclusive nature of the field. This lack of inclusivity and visibility has manifested as poor retention of BIPOC scientists coupled with discouraged individuals that encourage the next generation of scientists to flee academia. To face these challenges head on, BIPOC centered groups and organizations have been established to focus on addressing the needs and amplify the voices of their members. These groups aim to improve the overall experience of scholars within their institutions and encourage their progression

through the academic pipeline. In collaboration with other BIPOC groups, the Yale Black Postdoctoral Association (YBPA) will create a session geared towards understanding why these groups and organizations are necessary along with providing the tools to go about creating a successful venture. Beyond this, we will discuss what faculty and administrators can do to support the initiatives put forth by these groups and how to advocate for BIPOC postdocs as an ally.

**Postdocs and all research staff are supported and welcomed by Cushing/Whitney Medical Library**

**Kate Nyhan**, Dana Haugh

Cushing/Whitney Medical Library's research support services are available, for free, to postdocs and other research staff based at Yale School of Medicine, Yale School of Nursing, and Yale School of Public Health. Some 38 librarians and library staff offer literature, training, and consultations about bioinformatics, research data management, evidence synthesis, scholarly publishing, research impact, IACUC searching, and more. Contact your department's specialist liaison librarian to learn more about the library's offerings – or to suggest how we could serve postdocs' information needs better.

**Are We There Yet? Evaluating the Evidence for Neuroimaging-Based Biotypes of Psychiatric Vulnerability in the Acute Aftermath of Trauma**

**Ziv Ben-Zion**, Tobias R. Spiller, Jakob N. Keynan, Roei Admon, Ifat Levy, Israel Liberzon, Arie Y. Shalev, Talma Hendler & Ilan Harpaz-Rotem

**Objective:** The weak link between subjective symptom-based diagnostic methods for posttraumatic psychopathology and objectively measured neurobiological indices forms a barrier to the development of effective personalized treatments. To overcome this problem, recent studies have aimed to stratify psychiatric disorders by identifying consistent subgroups based on objective neural markers. Along these lines, a promising 2021 study by Stevens et al. identified distinct brain-based biotypes associated with different longitudinal patterns of posttraumatic symptoms. Here, the authors conducted a conceptual nonexact replication of that study using a comparable data set from a multimodal longitudinal study of recent trauma survivors.

**Methods:** A total of 130 participants (mean age, 33.61 years, SD=11.21; 48% women) admitted to a general hospital emergency department following trauma exposure underwent demographic, clinical, and neuroimaging assessments 1, 6, and 14 months after trauma. All analyses followed the pipeline outlined in the original study and were conducted in collaboration with its authors.

**Results:** Task-based functional MRI conducted 1 month posttrauma was used to identify four clusters of individuals based on profiles of neural activity reflecting threat and reward reactivity. These clusters were not identical to the previously identified brain-based biotypes and were not associated with prospective symptoms of posttraumatic psychopathology.

**Conclusions:** Overall, these findings suggest that the original brain-based biotypes of trauma resilience and psychopathology may not generalize to other populations. Thus, caution is warranted when attempting to define subtypes of psychiatric vulnerability using neural indices before treatment implications can be fully realized. Additional replication studies are needed to identify more stable and generalizable neuroimaging-based biotypes of posttraumatic psychopathology.

## **A maladaptive stress response promotes cell death during nutrient starvation**

**Todd Douglas**

Loss-of-function mutations in RBCK1 cause a fatal cardiomyopathy marked by glycogen accumulation in cardiac muscle. RBCK1 encodes HOIL-1, an atypical E3 ubiquitin ligase well known as an essential regulator of immunity but whose role in metabolism is undescribed. Here, using functional genomics, pharmacological interventions, and metabolomics, we found that loss of HOIL-1 activity dramatically alters cellular metabolism in cardiomyocytes. HOIL-1-mutant cells had impaired glycogen breakdown during acute glucose starvation, concomitant with the accumulation of cytosolic glycogen aggregates. Loss of HOIL-1 activity triggered a robust, feedforward antioxidant stress response during prolonged glucose starvation that causes noncanonical cell death, precipitated in part by cystine influx. HOIL-1-mutant cells were also hypersensitive to amino acid starvation-induced cell death. Using proximity labeling, we found HOIL-1 associates with stress granules during glucose starvation. Loss of the stress granule core protein G3BP1 attenuated cell death and stress responses during glucose starvation in HOIL-1-mutant cardiomyocytes. Ongoing work is focusing on examining cardiac function and metabolic stress in HOIL-1-null mice. Collectively, these data identify a previously unrecognized function of HOIL-1 in cellular metabolism and characterize a signaling cascade driving a noncanonical cell death program.

## **Blueprint for Community Emergency Department Pediatric Simulation Curriculum: From PECC to Practice**

**Snimarjot Kaur**, William Lynders, Michael Goldman, Christie Bruno, Juliana Morin, Scott Maruschock, Marc Auerbach

**Objectives:** Gaps in quality in pediatric emergency care have been noted in community emergency departments (CEDs), where >90% of children receive care. There is imminent need for interventions in low to medium-pediatric volume CEDs. Through this paper we aim to outline an innovative and replicable approach to develop CED pediatric-focused, in-situ simulation-based curriculum, facilitated by local pediatric champions, in remote collaboration with academic medical centers (AMC).

**Methods:** Kern's model; Problem identification/targeted needs assessment: review of recent pediatric transfer cases with leadership. Goals and objectives identified: pediatric education of the staff related to specific cases. Educational strategies: simulation + prelearning using podcasts, and videos and facilitated debriefing/resource sharing after simulations.

**Implementation:** 3-hour simulation sessions facilitated in person by local team and remotely by AMC. Leadership required participation + paid staff. Evaluation and feedback: retrospective pre-post survey, simulation effectiveness tool, net promoter score assessment, and qualitative discussions.

**Results:** 75 participants completed the post-session survey. Participants included 54.7% nurses, 22.7% physicians, and other staff. 34. 86.7% of the participants had cared for ≤ 10 critically ill pediatric patients in the past 2 years. 92% participants cited that simulation session was effective in teaching pediatric resuscitation skills. >72 % of the participants reported that the prebrief increased their confidence and >90% strongly agreed that debriefing was valuable in improving clinical judgment. Post simulation, participants reported better ability to appropriately evaluate critically ill newborns (14.7% pre vs. 64% post) and infants/toddlers (32% pre vs. 74.7% post). Participants strongly agreed that they can demonstrate more effective teamwork and better closed-loop communication (60% pre vs. 90.7% post). The Net Promoter Score was calculated to be 84%.

Conclusions: A locally facilitated in-situ simulation program was successfully implemented at a community-hospital under the leadership of the PECC with remote collaboration by AMC. The program was well received, effective, and scalable.

### **Genome-wide association study of alcohol consumption identifies novel loci in Latin American populations: Findings from the Latin American Genomics Consortium**

**José Jaime Martínez-Magaña**, Diego Andrade Brito, Rachel Kember, Hang Zhou, Henry Kranzler, Joel Gelenter, Sylvia Wassertheil-Smoller, Krista M Perreira, Martha Daviglus, Humberto Nicolini, Alma Delia Genis-Mendoza, Jorge Ameth Villatoro-Velazquez, Maria Elena Medina-Mora, Sintia Belangero, Marcos Santoro, Andre Rafael Simioni, Euripedes Miguel, Luis Rodhe, Rodrigo Bressan, Giovanni Salum, Pedro Pan, Katherine Tucker, Jose Ordovas, Latin American Genomics Consortium, Elizabeth Atkinson, Paola Giusti-Rodríguez, Janitza Montalvo-Ortiz

Heavy alcohol consumption is the major risk factor for alcohol use disorder. Genome-wide association studies (GWAS) are an excellent tool to study the genetic architecture of alcohol consumption, with recent substantial progress on identifying significant signals. However, most of the GWAS progress is mostly based on European populations; Latin American populations are still widely underrepresented. The inclusion of diverse populations in GWAS studies not only help improve power, but also would allow us to identify new loci potentially population-specific. In this study, we aimed to perform a GWAS of alcohol consumption in individuals of Latin American ancestry. We included 51 544 individuals from the Million Veteran Program (MVP), Hispanic Community Health Study/Study of Latinos (HCHS/SOL), Mexican Genomic Database for Addiction Research (MxGDAR), Brazilian High-Risk Cohort (BHRC), and Boston Puerto Rican Health Study (BPRHS). We analyzed AUDIT-C scores in MVP and the BHRC; for the BPRHS and MxGDAR we recoded drinks per occasion to the AUDIT-C question levels scores, and drinks per week in the HCHS/SOL. MVP was analyzed using linear regression adjusted for age, sex, and 10 principal ancestry components. HCHS/SOL, MxGDAR, and BHRC were analyzed using mixed linear models adjusted for age, sex, genetic relationship matrix, and the first 5 ancestry principal components. Lastly, all cohorts were meta-analyzed using random-effect models. We identified 15 genome-wide significant (GWS) single nucleotide polymorphisms (SNPs) associated with alcohol consumption in Latin American populations. 8 of these identified SNPs mapped to ADH1C. We also identified two GWS SNPs in the RAP1GDS1 gene also located in chromosome 4. 2 additional, and potentially novel, SNPs are located in the MTTP gene in chromosome 4. Our work identified well-known but also potential novel genetic associations with alcohol consumption in Latin American populations and adds to the increasing body of work diversifying alcohol-related GWAS studies.

### **A drug's most potent target is not necessarily the source of its anti-cancer activity**

**Debanjan Bhattacharjee**, Jaweria Bakar, Erin L. Sausville, Brianna E. Mendelson, Kaitlin Long, Joan C. Smith, Jason M. Sheltzer

The small-molecule drug ralimetinib (LY2228820) was developed as a p38 $\alpha$  (MAPK14) inhibitor and has advanced to phase 2 clinical trials in oncology. Thus far, ralimetinib has been tested in five different oncology trials but exhibited minimal clinical efficacy. Moreover, CRISPR-induced knockout of p38 $\alpha$  and p38 $\beta$  in cell lines fail to alter cancer cell sensitivity to ralimetinib, suggesting that its anti-cancer activity is independent of p38 $\alpha$ /p38 $\beta$  inhibition. This study applied a multi-modal approach to demonstrate that

ralimetinib's anticancer activity occurs due to its ability to inhibit EGFR, rather than p38 $\alpha$ . We found that mutant EGFR-driven cancer cell lines exhibit the greatest sensitivity to ralimetinib treatment, and ralimetinib phenocopies established EGFR inhibitors in pharmacogenomic profiling experiments. Furthermore, ralimetinib inhibited EGFR kinase activity in vitro and in cellulo, and a co-crystal structure revealed that ralimetinib functions as an ATP-competitive EGFR inhibitor. We also found that in an isogenic Ba/F3 model system, manipulating cells to rely on EGFR signaling for survival results in sub-micromolar sensitivity to ralimetinib. Finally, expression of the EGFR-T790M gatekeeper mutation was shown to confer resistance upon ralimetinib treatment. Our results illustrate how multiple complementary approaches, including CRISPR mutagenesis, pharmacogenomic profiling, targeted molecular assays, and structural evaluation, can be used to determine the MOA of an anti-cancer compound. Additionally, these results potentially explain the lack of therapeutic efficacy observed with ralimetinib in clinical testing. EGFR mutation status has not been used as a biomarker in any clinical trial with ralimetinib and these findings suggest that future clinical trials involving ralimetinib could incorporate EGFR mutation status as a biomarker to identify sensitive patients. Moreover, our results demonstrate that a compound's anti-cancer effects should not necessarily be attributed to the protein that it inhibits most strongly, and instead, comprehensive cellular and genetic profiling is required to understand a drug's mechanism-of-action.

### **Sex differences in the pleiotropy of hearing problems with imaging-derived phenotypes: a brain-wide imaging study**

**Jun He**, Brenda Cabrera-Mendoza, Gita A. Pathak, Dora Koller, and Renato Polimanti

**Background:** Hearing problems (HP) are one of the major health burdens in the elderly. While changes in the peripheral auditory system play an important role, genetic variation associated with brain structure and function could also be involved in HP predisposition.

**Methods:** We analyzed a large-scale HP genome-wide association study (GWAS; N=501,825, 56% females) and GWAS data related to 3,935 brain imaging-derived phenotypes (IDPs) assessed in up to 33,224 individuals (52% females). To investigate systematically HP pleiotropy with brain structure and function, i.e., loci affecting both traits, we conducted genetic correlation, latent causal variable (LCV), Mendelian randomization (MR), and multivariate logistic regression analyses. Additionally, we performed local genetic correlation and multi-trait colocalization analyses to identify genomic regions and loci implicated in the pleiotropic mechanisms shared between HP and brain IDPs.

**Results:** We observed a widespread genetic correlation of HP with multiple IDPs in the sex-combined analysis (Ntraits=171) and in females (Ntraits=120) and males (Ntraits=89), separately. Applying Bonferroni correction accounting for the number of IDPs tested, the LCV analyses showed that some of these genetic correlations could indicate cause-effect relationships. For seven of them, the possible causal effects were supported by an independent MR approach: vessel volume in the sex-combined analysis, hippocampus volume, cerebellum grey matter volume, primary visual cortex volume, and rfMRI-ICA100 node 46 in females, and global mean thickness and mean orientation dispersion index in superior corona radiata in males. The local genetic correlation analyses identified 13 pleiotropic regions of HP with respect to these seven IDPs. We also observed a colocalization signal for the rs13026575 variant between HP, primary visual cortex volume, and SPTBN1 transcriptomic regulation in females.

**Conclusion:** This study provides evidence that brain structure and function may have a role in HP predisposition via possible cause-effect relationships and shared regulatory mechanisms.

## **Seasonal variation in 5-HT<sub>1B</sub> receptor availability in healthy individuals**

**Waleed Ibrahim**, Takuya Toyonaga, Yanghong Yang, Marc N. Potenza, David Matuskey

**Objective:** To explore the significance of seasonal variation of 5-HT in healthy individuals using 5-HT<sub>1B</sub> PET imaging. **Background:** Here we used in vivo positron emission tomography (PET) imaging with the [<sup>11</sup>C]CE142 (aka – [<sup>11</sup>C] P943) tracer, that has increased sensitivity to 5-HT, to assess seasonal variations in relevant brain regions.

**Methods:** Sixty-six individuals (mean age (SD) = 30.1 (±9) years; males = 43, females = 23) participated in PET scans. BPND (binding potential non-displacement) values were calculated as the primary outcome measure for regions of interest including the raphe, anterior cingulate cortex, frontal lobe, putamen and caudate. The results were compared by applying 2-tailed t-tests to calculate group differences between months with high sunlight (April – September) vs. low sunlight (October – March). Correlations were also determined using the mean hours of sunlight exposure in New Haven, CT for each month and mean BPND values of the ROIs for each month.

**Results:** We obtained a statistically significant, higher BPND in raphe with high sunlight vs. low sunlight months (i.e., 0.532, 0.435; (p = 0.02)). However, there were no significant differences in the anterior cingulate cortex (0.986, 0.989; (p = 0.94)), frontal cortex (1.066, 1.053; (p = 0.74)), caudate (0.595, 0.623; (p = 0.51)) or putamen (1.035, 1.101; (p = 0.20)). The raphe also showed a significant negative correlation  $r = -0.6984$  (p = 0.01) with the amount of sunlight exposure, whereas all other regions were non-significant.

**Conclusion:** This is the first study to investigate seasonal differences in human brain 5-HT<sub>1B</sub> receptor availability. These findings reveal statistically significant results in the serotonin-rich raphe with amount of sunlight exposure in healthy individuals. The results suggest a role of the raphe in regulating circadian rhythm. Further, as 5-HT<sub>1B</sub> receptors have been implicated in MDD and SAD, the findings suggest a potential therapeutic link to SAD. Further studies involving SAD and 5-HT<sub>1B</sub> are warranted.

## **The interplay of testosterone and cortisol response to acute stress in the context of minority stress**

**Magdalena Mijas**, Ahmed Abdelfattah, Krzysztof Kasperek, Mateusz Blukacz, Karlina Koziara, Andrzej Galbarczyk, Urszula M. Marcinkowska, Grazyna Jasienska

While in men testosterone level often increases following psychosocial stress, this response is not universal and studies on individual variance of testosterone response to stress are still scarce. Previous research suggests that one of the factors contributing to the magnitude of testosterone response to stress is basal cortisol indicative of increased/prolonged exposure to stress and related HPA axis upregulation. None of the previous studies explored that relationship in the context of minority stress. In this study we aimed at investigating the interplay of testosterone and cortisol responses to acute stress in the context of chronic socially based stress (i.e. sexual minority stressors). 28 heterosexual men (HM group) and 15 gay and bisexual men (GBM group) were subjected to Trier Social Stress Test (TSST) and provided five saliva samples to determine baseline and post-TSST testosterone and cortisol levels. Multilevel mixed-effects REML-fitted regression models were used to investigate predictors of testosterone responses to stress and group differences (HM vs GBM) related to hormonal stress reactivity. Among GBM men sexual minority stressors were tested as predictors of hormonal responses to stress. Both baseline cortisol and its interactions with time significantly and negatively predicted testosterone responses to acute stress for the whole sample. Compared to HM group, GBM participants displayed limited testosterone response to stress as reflected by testosterone AUC<sub>i</sub> index. Additionally, among GBM participants vigilance associated

with sexual identity concealment and vicarious trauma positively predicted baseline cortisol. Our research indicate that baseline cortisol is associated with decreased testosterone stress reactivity. Observed differences between heterosexual and sexual minority men suggest that minority stress processes may have widespread physiological consequences in burdened populations.

### **Colocalization of phosphorylated TDP-43 with organellar proteins in TDP-43 positive AD patients**

**Aditi Naskar, Pallavi P. Gopal**

Transactive response binding protein (TDP-43) is a nuclear protein associated with transcription, mRNA splicing, miRNA processing, RNA metabolism during stress. In pathological condition it forms aggregates in the cytoplasm and known to be the hallmark of neurodegenerative disorders like amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration having ubiquitin inclusions (FTLD). Earlier studies showed that besides ALS & FTLD, cytoplasmic phosphorylated TDP-43 (pTDP-43) aggregates are also found in limbic predominant Alzheimer's disease. Dysfunctional endolysosomal system, proteasome system and impaired mitochondria are observed in AD characterized by A $\beta$  aggregates and tau tangles. In this study our aim is to identify the colocalization of phosphorylated TDP-43 (pTDP-43) with organelles that are involved in maintaining the homeostasis of the cell using stimulated emission depletion (STED). In this pilot study we have performed immunofluorescence for pTDP-43 and VDAC-1 (mitochondrial porin) on 5 $\mu$ m thick hippocampus sections collected from AD patients at autopsy from the Pathology brain bank. Our STED results shows a rim of mitochondria surrounding the p-TDP43 aggregates. Our future goal is to analyze more samples and also determine the colocalization of pTDP-43 with lysosomal and autophagy markers.

### **Chemokine producers reveal tissue-specific neutrophil niches**

**Alaz Özcan, José M Adrover, Sofía Vieto Fonseca, Andrés Hidalgo**

Once regarded as unsophisticated killing machines, neutrophils today are recognized for contributing to a variety of fascinating non-canonical functions, associated with tissue-specific signatures. Currently, the functional spectrum of neutrophils, their tissue-specific interaction partners, as well as the mechanisms behind their reprogramming are poorly understood. In this project, we embarked on an organism-wide expedition to illuminate tissue-specific neutrophil niches that instruct immune functions. We first conducted a thorough spatial characterization of CXCL1+ and CXCL12+ protein expression as well as neutrophil distribution, across 12 tissues, taking advantage of tissue-clearing techniques and confocal imaging. We then complemented these efforts with flow cytometry to identify the cellular sources of each chemokine. To distinguish in vivo interaction partners of neutrophils, we generated a neutrophil-specific lipoSoluble mCherry reporter (Neutrosberry) mouse line (inspired by Ombrato, Nat Protocols 2021). We determined that similar types of structural cells, including endothelial cells, pericytes, and fibroblasts, as well as certain specialized parenchymal cells i.e., keratinocytes, hepatocytes, and adipocytes, were responsible for homeostatic CXCL1 and CXCL12 production. The distribution of the cells producing either chemokine appeared to follow distinct anatomical programs that differed from tissue to tissue. Neutrophils displayed a clear predilection towards CXCL1 in the skin and appeared to exhibit an inflammatory program, unlike reparative lung neutrophils which resided preferentially in CXCL12+ niches. While the project is currently in its infancy, we have a clear roadmap to identify relevant functional programs associated with neutrophil niches and whether their remodeling underlies or synchronizes with

certain diseases, such as obesity, or impaired wound healing. By tracking normal or aberrant immune niches, we hope to gain invaluable insights into innate immune reprogramming and unveil functional hallmarks of inflammatory states.

### **Phosphoproteomic analysis of metformin signaling in colorectal cancer cells elucidates mechanism of action and potential therapeutic opportunities**

**Barbora Salovska**, Erli Gao, Sophia Müller-Dott, Wenxue Li, Carlos Chacon Cordon, George Rosenberger, Aurelien Dugourd, Julio Saez-Rodriguez, Yansheng Liu

The biguanide drug metformin is a safe and widely prescribed drug for type 2 diabetes. Interestingly, hundreds of clinical trials have been set to evaluate the potential role of metformin in the prevention and treatment of cancer including colorectal cancer (CRC). However, the “metformin signaling” remains controversial. To interrogate cell signaling induced by metformin in CRC and explore the druggability of the metformin-rewired phosphorylation network, we performed integrative analysis of phosphoproteomics, bioinformatics, and cell proliferation assays on a panel of 12 molecularly heterogeneous CRC cell lines. Using the high-resolute data-independent analysis mass spectrometry (DIA-MS), we monitored a total of 10,142 proteins and 56,080 phosphosites (P-sites) in CRC cells upon a short- and a long-term metformin treatment. We found that metformin tended to primarily remodel cell signaling in the long-term and only minimally regulated the total proteome expression levels. Strikingly, the phosphorylation signaling response to metformin was highly heterogeneous in the CRC panel, based on a network analysis inferring kinase/phosphatase activities and cell signaling reconstruction. A “MetScore” was determined to assign the metformin relevance of each P-site, revealing new and robust phosphorylation nodes and pathways in metformin signaling. Finally, we leveraged the metformin P-site signature to identify pharmacodynamic interactions and confirmed a number of candidate metformin-interacting drugs, including navitoclax, a BCL-2/BCL-xL inhibitor. Together, we provide a comprehensive phosphoproteomic resource to explore the metformin-induced cell signaling for potential cancer therapeutics.

### **Outcomes of the V-shaped noncoronary sinus remodeling technique for ascending aortic aneurysm and aortic root ectasia**

**Juan J. Velasco**, Hesham Ellauzi, Mohammad A. Zafar, Bulat A. Ziganshin, John A. Elefteriades

**Objective:** This study aims to evaluate the clinical outcomes of a novel V-shaped noncoronary sinus remodeling technique for the treatment of ascending aortic aneurysm and aortic root ectasia.

**Methods:** In this retrospective single-center study, 22 cases underwent a supracoronary V-shaped single sinus remodeling surgery in addition to aortic valve replacement (19), hemi-arch (9), and full arch with elephant trunk (2). The mean age was 62.4 years (SD 11.1). All patients had an ascending aortic aneurysm (mean, 4.6cm; SD, 0.5) and aortic root ectasia (mean, 4.4cm; SD, 0.3). Survival follow-up was 100% complete. The mean follow-up was 53 months (range 44.7 to 77) (SD 26.4).

**Results:** Hospital mortality was 0. The median hospital length of stay was 5.5 days (IQR 5-6). Survival in patients aged <60 was 100% at 24, 48, and 72 months; while in patients ≥60 it was 100, 91.6, and 91.6% (P = 0.56). No cases of thromboembolism or of postoperative aortic insufficiency were observed. Freedom from stroke or transient ischemic attack at 24, 48, and 72 months was 95.5%. The mean size of the aortic

root in the patients selected for the V-shape procedure was 4.4cm (SD 0.3). The early postoperative mean aortic root was 3.3cm (SD 0.5), while at last follow-up was 3.5cm (SD 0.6).

Conclusions: The V-shaped noncoronary sinus remodeling technique is safe and with excellent survival and freedom of major events. The V-shaped resection provides a good alternative accomplishing more than simply ignoring the moderately dilated aortic root but entailing less surgery than a Bentall (or other) fully resectional procedure.

### **Engineering of the high affinity chemokine CXCL13 to screen CXCR5 antagonists to treat cancer and autoimmune diseases**

**Manjula Ramu, Eric Rosenberg, Sam Katz, Francine Foss, Elias J. Lolis**

Autoimmune disorders and cancers are associated with aberrant activation of the CXCL13: CXCR5 signaling axis. AITL (Angioimmunoblastic T-cell lymphoma) is one such lymphoma, where activation of CXCR5 and its downstream signaling pathways may drive pathogenesis. CXCL13 is strongly expressed by dendritic cells within the spleen, lymph nodes, and Peyer's patches, where it binds to CXCR5 on mature B cells and TFH cells to induce B-cell development and differentiation and production of antibodies. Antagonists and Partial agonists for CXCR5 and antibodies against CXCL13 would act as potential drug molecules to reduce the severity of cancers and autoimmune conditions. In our study, we aimed to find an antagonist (small molecule/biotherapeutic) that can reduce the downstream signaling effect of CXCR5 in pathogenesis. We attempted to obtain biotherapeutics using CXCL13 protein engineering by modifying the N-terminal residues to produce partial agonists or antagonists. A phage display library of 168,000 random N-terminal variants was used to produce potential antagonists characterized by bioinformatic analysis. The most potent antagonist will be further validated for its ability to prevent cell migration and cancer cell survival by fusing to IgG and using in CAR-T therapy.

### **The taste of humans and nectar: gustation in the Asian tiger mosquito**

**Lisa Baik**

The *Aedes albopictus* mosquito is a highly invasive disease vector that is rapidly expanding its range. Mosquitoes use their taste system to guide important behaviors including feeding, biting, and egg laying, but the mechanisms by which the taste system controls these behaviors remain elusive. Here we examine how taste cues, simple and complex, are detected and discriminated by the taste system to drive behaviors of *Ae. albopictus* mosquitoes. We find that taste neurons of the major taste organ, the labellum, differentially encode ecologically relevant cues including nectar, sweat, and egg laying site water, responding with distinct neuronal profiles and subsequent behaviors. We identified 15 taste hairs called sensilla on the labellum. A systematic physiological screen revealed 3 major functional classes of taste sensilla, responding to several types of taste compounds including sugars, bitter compounds, salt, amino acids, and ammonia. Neuronal responses to tastants varied in magnitude and some tastants evoked strong excitation while others inhibition. We identified a subset of bitter compounds that inhibited physiological and behavioral responses to sugar, suggesting their use as potent stop signals against typically appetitive cues. Finally, transcriptomic profiling identified chemosensory genes expressed in the labellum, including gustatory and ionotropic taste receptors that likely underlie taste-driven behaviors. This dataset provides many molecular candidates that can be targeted to alter taste perception and behaviors. Our study sheds

light on key features of the taste system that may lead to new ways of manipulating chemosensory function and controlling mosquito vectors.

### **Quantification of Cardiac Amyloidosis from Tc-99m Pyrophosphate Planar Images Using a New Semi-automated Approach: Intra- and Inter-observability assessments in Patients**

**Divyani Goyal**, Veronica Sandoval, Christopher Weyman, Edward J Miller, Yi-Hwa Liu

Cardiac amyloidosis is a heart condition, where abnormal proteins accumulate in the heart. When these proteins build up, the heart struggles to pump, so it tries harder. Ultimately, the extra effort damages the heart, causing it to fail. There are various tests for diagnosing this disease, but there is a growing trend towards nuclear cardiac imaging in which a radioactive substance is injected into the blood, and uptake of this substance in the heart is measured as a ratio called H/CL ratio. The H/CL ratio is defined as the ratio of radioactive uptake in the heart to the uptake in contralateral lung. Derivation of H/CL ratio using this conventional manual processing method can be subjected to misinterpretation and thus can lead to misclassification of disease. This can have serious consequences as the disease is fatal, and treatment options are limited if diagnosed too late or overlooked. On the other hand, misclassification as positive for the disease can have economic consequences because Tafamidis, approved to treat this disease, is the most expensive cardiovascular drug ever sold in the United States. Therefore, it is important for such a crucial test to be reliable and accurate. We have developed a new method for calculating H/CL ratio. The new method uses an automatic region growing principle which may be less susceptible to the variability caused by conventional manual processing method. The study aimed to compare the inter- and intra-observer reproducibility of the new method with conventional method in calculating H/CL ratio. We hypothesize that H/CL derived from the new method may be less sensitive to noise and more reproducible as compared to conventional method. Inter- and intra- observer reproducibility were evaluated using Pearson's correlation coefficient and Bland Altman Plot. We demonstrated that intra-observer repeatability and H/CL values with the new method were higher.

### **Ssu72 mediated Pol II pausing at exon 2 is essential for co-transcriptional processing**

**Rajesh Kar**, A. Ansari, N. DeLanerolle

The C-terminal domain of RNA Pol II consists of conserved hepta-peptide repeats Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7. The post translational modification of these residues acts as a platform for recruiting various protein complexes that are involved in transcription initiation, elongation, splicing and termination. Various Kinases phosphorylates these repeats and phosphatases remove the phosphorylation marks from this to regulate various steps of transcription. In the genome wide studies of a phosphatase Ssu72, we observed an unusual enrichment at 3' splice site of intron containing genes, other than the known enrichment at TSS and CPS. As Ssu72 is an essential protein, we used the auxin induced degron tag to conditionally degrade it and found that it plays a key role in Pol II pausing at the 3'SS for co-transcriptional splicing. The more the intron length the more is the pausing of Pol II for proper splicing. In the absence of Ssu72 we observed the increase in Ser5 phosphorylation and acetylation at the second exon near the 3'SS that leads to increase in the transcripts.

### **Are the kinetics of EGFR clustering and ERK signaling connected?**

**Krishna C. Mudumbi**, Archer Hamidzadeh, Anatoly Kiyatkin, Mark. A. Lemmon

The extracellular signal-regulated kinase (ERK) is a member of the mitogen-activated protein kinase family and plays a critical role in cell growth, proliferation, and differentiation. The activation and signaling of this protein is dynamic and its kinetic control is key to determining cellular outcomes. Epidermal growth factor receptor (EGFR), a well known activator of ERK, has been previously shown modulate the kinetics of ERK signaling depending on which ligand – high or low affinity – activates EGFR to initiate signaling. However, it is unclear how this process might be regulated. Here we use various biochemical, fluorescence microscopy techniques to show that the activation of ERK might be directly tied to EGFR cluster size and clustering dynamics.

### **Teachers' intervention of conflicts between children: A cross-cultural comparison between Chinese and American early childhood educators**

**Zhenlan Wang**, Nicole Park, Fuzhe Xie, Craig S. Bailey

In this focus group study, we examined how early childhood educators intervene conflicts between children. We interviewed a total of 24 teachers from six different preschools and kindergartens in the U.S. and China. Our preliminary analyses found that teachers from both countries recognize the important role of emotion in conflict resolution. Whereas Chinese teachers scaffold perspective taking for the counterparts of the conflict, American teachers encourage children activate abstract empathy for the other side. Implications to education and future directions will be discussed.