

Keynote Speaker Abstracts

In order of presentation

Precision Oncology in the Pediatric Clinic: What Have We Learned?

Will Parsons, MD, PhD

Director, Center for Precision Oncology | Texas Children's Cancer Center and Baylor College of Medicine

Genomic sequencing technologies have facilitated the unbiased examination of cancer genomes, revealing the genetic landscapes of pediatric cancers and allowing rational prioritization of candidate genes and pathways for biological study and clinical application. Clinical experience with these methods has been limited, however, and the utility of “precision oncology” strategies for childhood cancer patients remains largely unproven. Over the past several years, a number of pilot studies of precision oncology have been conducted, demonstrating the feasibility of such approaches, providing early clues regarding the clinical utility (and limitations) of genome-scale testing, and emphasizing the critical importance of considering the impact of both tumor and germline results for patient care. Excitingly, these studies have provided a foundation for incorporation of genomic testing into prospective clinical trials utilizing biomarker-driven treatment assignments as well as the care of childhood cancer patients.

The Art and Science of Precision Medicine

Charmaine DM Royal, MS, PhD

Associate Professor, Departments of African & African American Studies, Biology, and Community & Family Medicine | Duke University

The Precision Medicine Initiative[®] (PMI) heralds a potentially transformative and far-reaching shift in the ways researchers and clinicians seek to understand and address health, disease, and health disparities. One of the most important (and challenging) components of the initiative is its proposed integrative approach to disease prevention and treatment informed by the effects of genes, environment, and lifestyle on disease risk. Despite the articulation and promotion of a holistic model, much of the rhetoric surrounding the PMI has focused solely on genomics. While the PMI was inspired by advances in genomics, it is the integration of genomic and other biological factors with contextual factors (e.g. social identities, socioeconomic status, cultural beliefs and practices, nutrition, physical activity, environmental exposures, psychosocial stress, discrimination, and healthcare access) that positions the initiative to revolutionize research and healthcare, radically improve individual and population health, and move us closer to achieving health equity. If precision medicine endeavors are to accomplish these goals, they must remain true to the guiding ecological scientific framework, while attending to the inevitable complex ethical and social issues such as community engagement, recruitment of diverse populations, informed consent, data stewardship and sharing, privacy and confidentiality, participant rights and welfare, return of secondary findings, and the blurring of boundaries between research and healthcare. This presentation will provide a brief background on the PMI and precision medicine more broadly, followed by an examination of issues related to the involvement of children in precision medicine efforts. Implications for various other stakeholders will also be discussed.

Oral Presentation Abstracts

In order of presentation

Personalized Hydroxyurea Dosing to Optimize the HbF response: Results from the TREAT Study

Patrick McGann, MD, MS

Cincinnati Children's Hospital Medical Center

BACKGROUND: Hydroxyurea is well established as the standard of care for children with sickle cell anemia (SCA), but current dosing schemes and dose responses for individual patients are highly variable. Traditionally weight-based dosing with careful and time-consuming dose escalation to maximum tolerated dose (MTD) results in an average hydroxyurea MTD of ~25 mg/kg/day and yields an average HbF of 20-25%. We developed and prospectively evaluated a population pharmacokinetics (PK)-based dosing model of hydroxyurea for children with SCA, aiming to initiate hydroxyurea at a personalized dose that achieves MTD quickly and achieves a more robust HbF response.

METHODS: The Therapeutic Response Evaluation and Adherence Trial (TREAT, ClinicalTrials.gov NCT02286154) is a prospective study with a primary objective to develop and evaluate a population PK-based model to predict the hydroxyurea MTD through an individualized dosing strategy. The study recruited patients (age 6 months to 18 years) who were initiating hydroxyurea therapy. We used a population PK model and a sparse sampling strategy to predict a starting dose that aimed to start at a dose that approximates MTD in order to optimize the benefits of hydroxyurea therapy.

RESULTS: Enrolled TREAT participants (n=38) were young with an average (mean±SD) age of 3.1±4.3 years and 50% of patients less than one year of age. The average starting dose was high (27.5±4.5 mg/kg/day), with 34% of starting doses ≥30 mg/kg/day. Despite the high starting doses, only 2 participants required a dose decrease from the starting dose due to moderate neutropenia. The individualized starting dose was the actual MTD for 73% of participants without the need for a single dose escalation. Even with high starting HbF (24.9±11.6%), response was excellent with all participants who have completed at least 6 months of hydroxyurea demonstrating an increase in HbF from baseline. The mean maximum achieved HbF is 35.5±9.5% with 74% achieving HbF>30% and 35% achieving HbF>40%.

CONCLUSIONS: TREAT has demonstrated the feasibility of individualized, PK-guided hydroxyurea therapy and suggests that early initiation of hydroxyurea for young children with SCA is both safe and effective, achieving HbF responses (>30-40%) beyond which has been seen with standard dosing.

Examining Heterogeneous States of Drug Responses of Cancer Cells by Microfluidic Sorting

Todd Sulchek, PhD

Georgia Institute of Technology

Assortments of cells may often consist of a heterogeneous populations of cell types that are desired to be isolated into important subsets, for example drug-responsive versus drug-resistant cells. The scarcity of these cell subpopulations makes it challenging to specifically study their cell biology or to develop countermeasures to potentially pathological subsets. We will discuss a label-free microfluidic sorting technology that can separate cells into biophysical subsets, including stiff and soft. The technology uses a microchannel decorated with repeated, diagonal ridges oriented skew to fluid flow. The interaction of these ridges with cells can be controlled to achieve the desired sorting properties. As a proof of concept, we show how the device can be used to analyze molecular distinctions between drug-resistant and drug-sensitive cells. Drug-sensitive cells were found to be apoptotic and over twice as stiff as drug-resistance cells and therefore could be separated with high accuracy. Consequently, the role of multiple mechanisms of drug resistance could be identified through molecular analyses of the sorted subpopulations of cells. We found several mechanisms of drug resistance, including decreased sensitivity to apoptosis, enhanced metabolism and extrusion of drugs, and, for the first time, the role of estrogen receptor in drug resistance of leukemia cells. Other applications of ultrafast compressions on cells will be discussed.

Chemosensitizing Solid Tumors to DNA Damaging Chemotherapy via Polymer-Mediated RNA Interference

Erik C. Dreaden, PhD

Georgia Institute of Technology & Emory University School of Medicine

Precision medicines have greatly improved treatment outcomes in both pediatric and adult cancer patients; however, small molecule- and protein-based therapeutics are capable of drugging only a small fraction proteins implicated in cancer pathogenesis or progression. RNA interference (RNAi) therapies, in contrast, can selectively deplete nearly any disease-causing protein and, while exciting, their systemic delivery to-date remains challenging. Here, I'll describe recent progress from our laboratory in the synthesis and preclinical development of an amphiphilic delivery vehicle for small interfering RNA that sensitizes primary and metastatic tumors to frontline chemotherapy in vitro and in vivo. We find that RNAi-mediated loss of the kinase, MK2, is synthetic lethal in combination with p53 deficiency in solid tumors and that nanoparticle-mediated silencing of the checkpoint kinase dramatically sensitizes tumors to frontline chemotherapy currently used in the clinic. We further identify a new genetic dependency between MK2 and the nucleotide excision DNA repair (NER) pathway and exploit this finding to simultaneously co-silence MK2 and the critical NER factor, XPA, as a means to further sensitize tumors to DNA-damaging chemotherapy and prolong survival. This work demonstrates how polymer nanotechnologies can exploit new insights in cancer cell signaling to address unmet needs in current clinical interventions for cancer and other diseases.

The Biorepository and Integrative Genomics (BIG) Initiative at Le Bonheur Children's Hospital

Robert Rooney, PhD

University of Tennessee

While precision medicine holds great promise for better and more cost-effective care, there is also potential to create additional health care disparities if it remains largely unavailable in traditionally underserved communities. The Memphis metropolitan statistical area is the most impoverished and largest metropolitan area with an African American-majority population in the United States. Le Bonheur Children's Hospital (LBCH) is the sole pediatric tertiary-care center in this 10 county area, with 380,000 encounters annually at its inpatient, emergency department and outpatient facilities. The Biorepository and Integrative Genomics (BIG) Initiative at LBCH and the University of Tennessee Health Science Center (UTHSC) represents the first steps toward introducing precision medicine to the Memphis community, initially as a means to facilitate genomics research and to develop the infrastructure for precision medicine platforms at LBCH and other Methodist/Le Bonheur Healthcare facilities.

BIG created a governance structure with committees to supervise planning and operations, ethics, community outreach and research oversight, attracting widespread participation from LBCH, CFRI and UTHSC department, division and facility heads. Since starting in 2015, BIG has: 1) developed an enrollment process to approach the majority of LBCH pediatric patients for consent to isolate, store and analyze their genomic DNA; 2) created and consolidated informatics systems with EMR integration for HIPAA-compliant participant sample and data management, linkage of samples to de-identified clinical information, and on-line sample and data requests by UTHSC researchers; and 3) constructed an operational pipeline to extract, archive, and distribute for analysis germline DNA from blood leftover in blood draws ordered for standard of care testing. Construction of facilities for clinical genomic analyses is currently under way.

As BIG has grown, enrollment approaches 150 participants per week from the hospital's inpatient and outpatient center populations, with a 78% consent rate. Currently, BIG has enrolled >8,000 participants and archived >4,400 individual DNA samples, with representative coverage of the demographics and medical issues in the Memphis pediatric patient population. We believe that BIG can serve as the basis for developing precision medicine platforms at LBCH, as well as facilitating genomic research that can benefit the Memphis area community.

Modeling Familial Dysautonomia Using Human Pluripotent Stem Cells

Nadja Zeltner, PhD

University of Georgia

Functional and molecular aspects of human genetic disease can be recapitulated in vitro using patient-specific, human induced pluripotent stem cells (iPSCs). Familial Dysautonomia (FD) is a debilitating developmental and degenerative disorder with onset at birth that primarily affects derivatives of the neural crest (NC), particularly the peripheral nervous system (PNS). Symptoms are

defective sensation of pain, spine deformations and the inability of the sympathetic nervous system to regulate stress. For unknown reasons, FD patients present with mild or severe disease symptoms despite carrying the identical, homozygous point mutation in *IKBKAP*.

We used iPSCs derived from FD patients and differentiated into various PNS cell types to present phenotypes at various stages of development that capture severe and mild FD symptoms. Patient-specific cells only from severe but not mild FD display an impaired capacity of developing into NC derivatives, such as autonomic and sensory neurons, thus they have neurodevelopmental defects. Interestingly however, both severe and mild FD cells show defects in peripheral neuron survival, indicating neurodegeneration as the primary culprit in mild FD. Importantly, we found that neuronal degeneration in mild FD can be halted by treatment with candidate therapeutic compounds kinetin and SKF-86466. Genetic rescue of the FD mutation in severe FD iPSCs reversed NC, but not sensory neuron lineage phenotypes, implicating that the known FD mutation does not account for all symptoms. Employing whole-exome sequencing (WES), we identified candidate mutations that were only found in severe but not mild FD patients, providing evidence that FD may constitute two genetic sub-diseases. Our study demonstrates that human iPSC-based disease modeling is sensitive in recapitulating disease severity. This paves the road for applications in personalized medicine and raises the prospect that individual patient's disease could be studied in vitro.

Screening a library of small molecules, we further identified a novel compound that rescued severe FD defects. This compound is the active chemical in a Traditional Chinese Medicine, making it an interesting possible treatment option for preventing neurodegeneration in FD and possibly more common peripheral neuropathies. This further paves the way for future treatments tailored more specifically towards individual patients.

3D Bioprinted Vascular Cardiac Tissue as a Platform to Study Congenital Heart Defects

Vahid Serpooshan, PhD

Georgia Institute of Technology & Emory University School of Medicine

Studying pathophysiological mechanisms of congenital cardiovascular disease necessitates bioengineering of new platforms that can accurately simulate the intricate microenvironment of the developing heart. Genetically engineered animal models often diverge from the human phenotype and 2D in vitro models also fail to recapitulate the phenotypic-functional features of in situ heart. 3D bioprinting and perfusion-bioreactor technologies have emerged as new tools that enable dynamic, 3D culture of tissue constructs that mimic the in vivo structural, molecular, and mechanical complexity. These technologies allow for precise spatial and temporal control of cells as well as environmental exposures of these tissues to variations in blood flow and metabolic perturbations. In this study, we hypothesize that recapitulating physiologic vascular architecture and flow in bioprinted constructs provides a unique in vitro platform to study heart development and congenital heart disease. Our goal is to create patient-specific 3D myocardial tissues and determine the effect of vascular design and flow on cell viability and function. Our results demonstrate the feasibility of

printing viable and functional human iPSC-derived cardiomyocytes (hiPSC-CMs) and endothelial cells (ECs) using gelatin methacrylate (gelMA) as bioink. hiPSC-CMs were encapsulated in gelMA (2M/mL) and printed into a perpendicular mesh network and the negative space was used as vasculature. To form endothelium onto the printed channels, we used a custom-printed bioreactor to perfuse ECs (10M/mL) into the microchannels. Optimal flow conditions and design parameters to achieve hiPSC-CM viability, function, and maturation were identified. We are now utilizing this platform to study Hypoplastic left heart syndrome (HLHS), a severe congenital heart disease that is associated with underdeveloped left ventricle. While previous studies had shown an intrinsic deficiency of HLHS hiPSCs in cardiac differentiation, here we showed efficient cardiac differentiation and significant viability (>80% CMs). Further, to investigate the role of “no flow, no grow” in HLHS pathogenesis, we utilize our bioprinted platform to perfuse 3D tissue constructs, consisting of control (healthy) or HLHS iPSC-CMs, at varying flow rates. Together, outcomes of this research will establish the proof-of-concept for the first generation of bioprinted, patient-specific, vascular platforms that can be broadly applicable to in vitro modeling of various cardiovascular diseases.

Poster Presentation Abstracts

1. Human B cell Responses to a Live Attenuated Cholera Vaccine

Adekunle, Oluwaseyi; Kauffman, Robert; Cho, Alice; and Wrammert, Jens

Vibrio cholerae is a non-invasive gut mucosal bacterial pathogen that causes cholera, a severe acute diarrheal disease. It infects millions of people each year and is responsible for 100,000 deaths annually. The currently available inactivated whole cell cholera vaccines do not provide durable long term protection. In contrast, natural infection with *V. cholerae* generates long term protection. The precise mechanism of protection is unknown, although B cells are thought to play an important role. Given the burden of cholera disease, there is a need to develop more effective and durable vaccines. Recently, a live attenuated cholera vaccine (Vaxchora) was approved for use in the US. Vaccination with a live attenuated cholera vaccine may elicit immune responses more similar to natural infection. Comparative analysis of vaccination and infection induced immune responses could highlight differences that are important for protection. To this end we have initiated a study to characterize B cell responses generated in individuals vaccinated with a live-attenuated cholera vaccine. These findings will be compared to ongoing studies of immune responses to natural infection. Preliminary results from the study participants has shown that the vaccination-induced plasmablast response peaked on day 7. Additionally, these responses were specific for cholera toxin and lipopolysaccharide, the two major immunodominant antigens. A large percentage of the plasmablast population expressed the gut homing receptor CCR9 which may indicate homing to the mucosa. The serological responses were similar in specificity and kinetics to the plasmablast response, and demonstrated the induction of vibriocidal and agglutinating antibodies. These preliminary findings demonstrate the use of the live attenuated cholera vaccine as an effective tool

for examining systemic and mucosal immune responses, both for cholera and potentially other mucosal pathogens.

2. Chemical Modification of Pediatric Tubing for Antibacterial Function

Aioub, Allison; Geng, Zhishuai; Kim, Jenny; Zheng, Leo; Garren, Mark; Beveridge, Jennifer; Chenot, Haley; and Finn, M.G.

To combat the growing rate of secondary infections in indwelling medical devices, we have developed methods to covalently modify medical grade polyvinyl chloride-based catheter tubing with antimicrobial agents. We first optimized the replacement of chloride with azide groups in the outer layer of the PVC material, followed by coupling of the resulting azides with various compounds known to have cytotoxic or barrier effects against clinically-relevant bacteria. The surface morphology and hydrophobicity of the resulting tubing was changed considerably, but considerable mechanical flexibility was retained due to the retention of plasticizer in the bulk of the material. The attachment of hydrophobic quaternary ammonium compounds was shown to provide excellent contact toxicity against both Gram-positive and Gram-negative bacteria, but to be nontoxic toward mammalian cells. Synthetic, analytical, and in vitro functional results will be described.

3. Specific MicroRNAs Present in Matrix Vesicles Regulate Growth Plate Chondrocyte Proliferation and Maturation

Asmussen, Niels C.; McClure, Michael J.; Lin, Zhao; Schwartz, Zvi; and Boyan, Barbara D.

INTRODUCTION: Local cell communication regulates the proliferation and differentiation of chondrocytes within growth plate cartilage during endochondral bone formation, culminating in extracellular matrix (ECM) mineralization. Chondrocytes are responsible for the synthesis and maturation of calcifiable ECM. Within this ECM are matrix vesicles (MVs), which are selectively enriched with enzymes and growth factors. We recently discovered that MVs are also selectively enriched with microRNAs (miRNA). The aim of this study was to determine whether specific miRNA that are enriched in MVs regulate growth zone (GC) chondrocytes.

METHODS: GC cells from the costochondral cartilage harvested from 5-week-old 125g male Sprague Dawley rats were isolated and cultured. Passage four GC cells were transfected with miRNA mimics (miR-22, miR-122, miR-223, miR-451). Cell layers and conditioned media were harvested. All experiment groups had an n of 6 cultures per variable. A one-way analysis of variance with Tukey's multiple comparison test was carried out on the data. All significant differences have a $p < 0.05$ and were determined using Prism v 5.04 and R v 3.4.3. Results were validated in repeat experiments.

RESULTS: DNA synthesis increased in GC cultures transfected with miR-122 while other miRNA had no effect. Cell layer alkaline phosphatase specific activity was reduced by miR-122, whereas

sulfated glycosaminoglycan (sGAG) content was increased. OPG production was increased by miR-122; RANKL was undetected for all groups; and collagen 2 and VEGF were increased by miR-22.

DISCUSSION: Taken together, our data indicate that miRNAs enriched in MVs are active components in the regulation of chondrocyte proliferation, differentiation, and ECM synthesis. MicroRNA-122 appears to be driving chondrocytes to remain in the proliferative stage of the growth plate by acting to delay chondrocyte maturation and increasing cellular proliferation. Understanding the role that various miRNAs play in the maturation of chondrocytes in the growth plate may be invaluable in addressing the multitude of skeletal dysplasias that can affect children. The potential to regulate growth plate chondrocytes through miRNAs and their inhibitors presents a possible novel method to address developmental disorders.

4. The SIX1 Homeobox Gene is a Novel CRM1-Dependent Target in CALM-AF10 Leukemias

Aumann, Waitman; Lavau, Catherine; Conway, Amanda; and Wechsler, Dan

BACKGROUND: The CALM-AF10 translocation is detected in 5 to 10% of T-cell acute lymphoblastic leukemias (T-ALLs), and some acute myeloid leukemias (AMLs). CALM-AF10 leukemias, similar to Mixed-Lineage-Leukemia (MLL) leukemias, are characterized by high expression of proleukemic HOXA genes. Since HOXA genes are difficult to target, we hypothesized that identification of non-HOXA CALM-AF10 effector genes could yield novel therapeutic targets. We took advantage of our prior observation that the nuclear export factor CRM1/XPO1 tethers CALM-AF10 to HOXA genes by interacting with a nuclear export signal (NES) in CALM. Using RNA-sequencing and microarrays, we found expression of SIX1, a homeobox gene highly expressed during embryogenesis, is increased in CALM-AF10 leukemias and decreased in response to Leptomycin B (LMB), a CRM1 inhibitor.

DESIGN/METHODS: RT-qPCR was performed on bone marrow progenitors and murine embryonic fibroblasts (MEFs) transduced with CALM-AF10, with and without Leptomycin B. Chromatin Immunoprecipitation (ChIP) was performed on CALM-AF10 transduced MEFs. CALM-AF10 transduced fetal liver progenitors were used in methylcellulose colony assays to examine SIX1's ability to enhance self-renewal.

RESULTS: Similar to HOXA genes, RT-qPCR confirmed overexpression of SIX1 in both CALM-AF10 transduced MEFs and CALM-AF10 leukemias. In addition, decreased SIX1 expression was observed in the presence of LMB. ChIP revealed that CALM-AF10 binds the SIX1 locus. Overexpression of SIX1 in fetal liver cells was sufficient to increase the self-renewal potential of colony forming progenitors.

CONCLUSIONS: SIX1 is a homeobox gene that is highly expressed during embryogenesis and is involved in the epithelial mesenchymal transition (EMT) and organogenesis. While SIX1 expression is silenced post embryogenesis, increased expression has been reported in numerous solid tumors; however, a potential role for SIX1 in leukemias is less clear. We have now determined that SIX1 is upregulated in the presence of CALM-AF10, and that SIX1 increases the self-renewal potential of

hematopoietic progenitors. These observations indicate that SIX1 may play a pathogenic role in leukemogenesis, and that SIX1 could be a novel therapeutic target in CALM-AF10 leukemias. The fact that SIX1 is not expressed post-embryonically suggests that inhibition of SIX1 might be effective in impairing CALM-AF10 leukemia cell proliferation, with few off-target effects.

5. Formulation and Characterization of Novel Particulate Vaccine Against Gonorrhoea

Bajaj, Lotika; Gamal, Wael; Gala, Rikhav; Zughair, Susu; and D'Souza, Martin

INTRODUCTION: Gonorrhoea is one of the most common sexually transmitted infections caused by Gram-negative Diplococcus bacteria, *Neisseria gonorrhoea*. The treatment for gonorrhoea involves use of antimicrobials but development of drug resistance is a great threat to public health and therefore novel methods for prevention of gonorrhoea infection are needed.

METHODS: In the present study, we formulated spray dried microparticles with pre-crosslinked BSA to deliver and evaluate efficacy of formalin fixed whole cell of gonorrhoea bacteria as vaccine. The microparticles were characterized for size, charge and poly dispersity index (PDI). In-vivo efficacy of this vaccine, was checked in 4-6 weeks old Balb/c mice. One group received subcutaneous gonorrhoea microparticulate vaccine (GnH MP), second received subcutaneous gonorrhoea vaccine in suspension and third received blank BSA microparticles (N=6). Blood samples were collected every 2 weeks after dosing and antibody levels were measured using indirect ELISA for IgG antibodies.

RESULTS: The percent yield for vaccine particles was 89 % w/w. Vaccine particles were 4.5 μ m and PDI was 0.447 with a charge of -25.1 ± 5.79 mV. An increase in specific antibody levels was observed in mice beginning at week 4 in particulate vaccine groups.

CONCLUSION: Vaccine particles were prepared and in-vitro and in-vivo characterization was successfully done.

TRANSLATIONAL IMPACT: This vaccine shows promise since the whole cell vaccine can be used without modifications in humans after appropriate scale up. By using the whole cell bacteria, all the antigens are preserved.

6. Evaluation of the In Vivo Efficacy of Microencapsulated β TC-6 cells in Diabetic Mice for the management of Type I Diabetes Mellitus

Bansal, Amit; D'Sa, Sucheta; and D'Souza, Martin

Type 1 diabetes mellitus (T1DM) is a disease characterized by lack of pancreatic islet function. Whole tissue transplantation appears to be a viable alternative in the management of T1DM due to the limitation of exogenous insulin therapy. This study aims at fabrication and evaluation of alginate-chitosan microcapsules encapsulated insulin secreting β TC-6 cells using specialized

spraying nozzle. Microcapsules encapsulated with β TC-6 cells were fabricated using novel spraying device producing uniform spherical microcapsules. Microcapsules were characterized for permeability using molecular weight markers, stability, and cell viability using Live Dead Staining Kit. Microencapsulated β TC-6 cells were transplanted intraperitoneally in streptozotocin (STZ) induced diabetic mice and monitored for lowering in blood glucose level and immune acceptance. Spherical microcapsules with a diameter in the range of 250-350 μm were prepared at an air flow rate of 250 L/Hr. Microencapsulated β TC-6 cells in alginate capsules demonstrated prolong viability. The group received microencapsulated β TC-6 cells maintains normoglycemia for 35 ± 5 days before rejection. However, the group received naked β TC-6 cells rejected graft within 1 or 2 days and exhibit both cellular and humoral immune responses. CD4 T-cells mediated Th2 response i.e. humoral response was predominant in microencapsulated β TC-6 cells group and that was further confirmed by elevated levels of CD45R. Microcapsules produced by specialized nozzle were reproducible with narrow size distribution and in addition, provides flexibility in producing different sized capsules. Our findings for in vivo study revealed that transplantation of microencapsulated β TC-6 cells may be a viable alternative in the management of T1DM with greater immune acceptance.

7. Evaluation of the Efficacy of Cationic pDNA Nanovaccine for Immuno-contraception and Rabies Control

Bansal, Amit; Wu, Xianfu; and D'Souza, Martin

Plasmid DNA (pDNA) vaccines have the potential to elicit an immune response against a wide range of diseases. However, the limitation of poor uptake of pDNA to antigen-presenting cells and rapid degradation of pDNA encapsulated in nanoparticles prompted us to fabricate an encapsulation free pDNA nanoparticulate vaccine. The negatively charged pDNA adsorbs on cationic PLGA (poly (d, l-lactide-co-glycolide))-chitosan nanoparticles and were used as means to deliver pDNA.

Nanoparticles in the size range of 380-500 nm were prepared using emulsification method. The binding affinity of pDNA to cationic nanoparticles depends on pDNA to nanoparticles ratio (P/N) and complete immobilization of pDNA to cationic nanoparticles was achieved at a P/N ratio of 1/50. In vivo efficacy was determined in mice administered with a prime dose and two booster doses at week 4 and 8. Serum was collected every week up to 12 weeks post vaccination and analyzed for gonadotrophin-release hormone (GnRH, a reproductive hormone) specific antibody. The binding of the GnRH specific antibodies was measured by avidity elution ELISA.

pDNA adsorption efficiency of 99.0 percent was achieved in pDNA PLGA-chitosan nanoparticles prepared using chitosan glutamate concentration of 2 mg/mL. Complex of pDNA and cationic nanoparticles was well tolerated and maintained cell survival rate greater than 80.0 percent.

Additionally, cellular uptake was found to be both time and concentration dependent and followed saturation kinetics with V_{max} of 11.389 $\mu\text{g}/\text{mL}\cdot\text{hr}$ and K_m value of 139.48 $\mu\text{g}/\text{mL}$. In vitro release study of P/N, 1/50 showed that the nanoparticulate vaccine can sustain the release of pDNA up to 24 hours. In vivo, elevated levels of GnRH specific IgG, IgG2a antibody response was observed in

mice administered with pDNA nanovaccine and adjuvant (Alum and MF59) compared to mice administered with pDNA and blank nanoparticles. Serum antibody avidity was also found to be highest in mice administered with pDNA nanovaccine and adjuvant at week 9 and 12.

CONCLUSION: In our study, we demonstrated that pDNA PLGA-chitosan nanoparticles were non-cytotoxic, showed enhance cellular uptake, and sustain the release of pDNA for an extended period. Generation of GnRH specific antibodies confirms the potential of nanovaccine to induce immunocontraception and spread of rabies virus.

8. Acute Lung Injury in Sickle Cell Mice from Effects of Complement Activation

Bell, Gregory V.; Patel, Seema R.; Mener, Amanda; Bennett, Ashley M.; Archer, David R.; Joiner, Clinton H.; Stowell, Sean R.; and Chonat, Satheesh

Sickle Cell Disease (SCD) results from a genetic mutation in beta-globin, where amino acid valine replaces glutamic acid. The resulting abnormal hemoglobin on deoxygenation causes red blood cells (RBCs) to sickle, become dehydrated, less deformable, and abnormally adhesive with greatly shortened lifespan. Repeated sickling and chronic hemolytic anemia lead to parenchymal injury, chronic inflammation, and endothelial dysfunction, which ultimately results in substantial morbidity and early mortality. Acute Chest Syndrome (ACS) is a serious complication seen in patients with SCD and remains a leading cause of hospitalization and the most common cause of death due to SCD. Patients with ACS present with a combination of fever, cough, chest pain and symptoms of respiratory distress, along with pulmonary infiltrates on the chest x-ray. Currently, clinical management of ACS relies exclusively on analgesics, antibiotics, red cell transfusions and respiratory support. Understanding the complex pathophysiological mechanisms driving ACS is a critical need. We hypothesize that alternative complement pathway (ACP) activation contributes to hemolysis and the pathogenesis of acute lung injury (ALI) in SCD. We formulated this hypothesis on the basis of our recent discovery that injection of cobra venom factor (CVF), a commonly used approach to induce complement activation, to sickle mice results in acute hemolysis, rapid deoxygenation, hypopnea, and bradycardia consistent with ACS. All these sickle mice die within 2 hours of CVF treatment. In contrast, none of the littermate control mice developed significant hemolysis, pulmonary compromise or succumbed to CVF administration. Our data suggest that systemic activation of complement is involved in the pathogenesis of ALI in mice. Rapid drop in hemoglobin, release of free heme, production of complement anaphylatoxins (C3a and C5a) and, membrane attack complex (C5b-9) would be predicted to facilitate neutrophil activation and endothelial activation/injury, and ultimately leading to ACS in patients with SCD.

9. The Relationship Between Microglia Activation and Sickle Cell Disease in Mice

Bell, William; Rubaharan, Myu; and Murphy, Anne

Sickle Cell Disease (SCD) is a genetic disease that results in an abnormal hemoglobin structure in red blood cells. Under normal conditions, a red blood cell will be disc shaped and flexible as it moves through the body. SCD results in the formation of stiff rod shaped hemoglobin causing the

shape of the cells to resemble a sickle, making it far more difficult to move through veins and capillaries and resulting in a higher likelihood of blockages known as Vaso Occlusive Crises (VOC). VOC prevent blood flow through the blood vessels and are characterized by intense sometimes chronic pain. Pain responses, similar to those symptomatic of SCD, have been observed in the periaqueductal gray (PAG) through morphological changes in microglia cells that are indicative of an increased level of activation that in turn indicates neuroinflammation. Per Gupta et. al, 2015, mast cell Toll-Like Receptor 4 (TLR4) inhibition via TAK242 in sickle cell mice with chronic pain showed a significant decrease in the release of tryptase (indicator of mast cell activation), substance-P (neurotransmitter associated with pain/inflammation), and IL-6 (a proinflammatory cytokine) indicating that therapies targeting TLR4 inhibition may be beneficial in alleviating sickle pathobiology. The role of the PAG in pain response remains somewhat unclear, but due to the level of chronic pain response associated with SCD we have hypothesized that microglial cells will exhibit a level of increased activation in SCD mice as opposed to control mice. There is growing evidence suggesting a cross talk between mast cells and microglia. However, little is known of the microglial TLR4's role in SCD (Giusti et al, 2013). We aim to investigate microglial TLR4 activation in chronic pain response observed in SCD.

10. Clinical Utility of Targeted RNA-Sequencing in the Diagnosis of Neuromuscular Disorders

Berger, Kiera; Chakravorty, Samya; Arafat, Dalia; Shenoy, Sreekala; Gibson, Greg; and Hegde, Madhuri

BACKGROUND: As therapies and clinical trials for individual Neuromuscular Disorders (NMD) increase in availability, it becomes increasingly important to provide a specific diagnosis for each case. Due to the heterogeneity of NMD, particularly muscular dystrophies and myopathies, diagnosis is often reliant on identification of pathogenic variant(s) via DNA sequencing of multi-gene panels. While Emory Genetics Laboratory has shown a diagnostic yield near 50% using a targeted NMD gene panel, a high prevalence of variants of unknown significance (VUS) limits improvement. We predict transcriptomics will increase the diagnostic yield by providing the functional evidence necessary to classify these variants. In addition, we hope to use gene expression analysis to identify skeletal muscle genes that are consistently over- or under-expressed in specific disorders or NMD in general.

METHODS: RNA was extracted from patient muscle biopsies and subjected to targeted RNASeq with a panel of 273 known neuromuscular disease genes. Following alignment of Illumina paired-end 150bp sequences, a tiered analysis pipeline involving variant calling with GATK, estimation of exon- and gene-specific abundance with DEXSeq and DESeq respectively, and calculation of percentage splicing with MISO as well as data visualization with Sashimi plots, was introduced to identify likely pathological changes in gene expression.

RESULTS: For each of 15 patients, a candidate gene or genes had been identified based on DNA sequencing and clinical presentation. RNA sequencing detected variants in the candidate gene(s) at more than 50X read depth in all but 2 patients, illustrating that the targeted gene panel provides the resolution needed to confidently identify functional effects. Initial analysis led to novel definitive

diagnoses for two samples, one of GNE Myopathy and another of Duchenne Muscular Dystrophy. Early trends seen in gene expression analysis suggest a significant portion of skeletal muscle genes may be differentially expressed in NMD cases, indicating that a larger number of samples could help define expression profiles for individual disorders or lead to identification of biomarkers for NMD.

CONCLUSIONS: Targeted RNA-Seq from muscle biopsies has great potential to increase the diagnostic yield of the genetic basis for neuromuscular diseases and simultaneously validate predictions from DNA sequencing.

11. Doxorubicin Conjugated to Reovirus Enhances Viral Oncolysis of Triple-Negative Breast Cancer Cells

Berry, Jameson; and Mainou, Bernardo

Treatment of triple-negative breast cancer (TNBC), which lacks expression of estrogen and progesterone receptors and HER2, is largely limited to cytotoxic chemotherapy. One approach to increase treatment efficacy is combination therapy. Reovirus is an oncolytic virus which preferentially infects and kills transformed cells. In a small molecule inhibitor screen, doxorubicin hydrochloride robustly enhanced reovirus infectivity while significantly reducing the number of MDA-MB-231 TNBC cells. Doxorubicin is a topoisomerase II inhibitor that causes DNA double-strand breaks and cell death. To circumvent off-tumor cytotoxic effects of the drug, doxorubicin-conjugated reovirus (reovirus-dox) will be assessed for increased reovirus cytopathic efficacy with enhanced selective delivery of doxorubicin to TNBC cells. Preliminary results indicate that reovirus-dox attachment to MDA-MB-231 cells is slightly diminished. Reovirus-dox establishment of infection is impaired, but replication kinetics are unaltered. Additionally, the levels of doxorubicin conjugated to reovirus positively correlate with overall cytopathicity and faster induction of apoptosis than parental virus. Future studies will define the mechanisms of reovirus-dox-induced cell death and test selective delivery of doxorubicin to TNBC cells. Conjugation of small molecule inhibitors to reovirus may provide an effective new method to directly target and kill cancer cells in a multi-faceted combinatorial approach.

12. Heart of Culturing: Requiring an Indication for Ordering Cultures in a Cardiac Service Line

Bloch, Deborah; Gruenewald, David; Carter, Alexis B.; Gonzalez, Mark D.; Chanani, Nikhil K.; and Shane, Andi L.

BACKGROUND: Ordering unnecessary cultures increases the probability of clinically insignificant results leading to unnecessary antibiotics and resource utilization. We evaluated whether requiring providers to choose a single clinical indication for culture at the time of order in an Electronic Health Record (EHR) in a tertiary pediatric healthcare system inpatient cardiac service line impacted ordering practices and subsequent clinical decision making.

METHODS: The culture indications requirement was implemented in March 2016. Only one option could be selected from a list of indications, which included: temperature instability, hemodynamic instability, abnormal white blood cell count, increased C-reactive protein, and other

with option for free text. EHR abstraction of culture orders, indications and results was performed for the pre-implementation (January 2015 - February 2016) and post-implementation (March 2016 - July 2017) periods. Single episode of *Staphylococcus epidermidis* containing cultures were considered clinically insignificant. User satisfaction was assessed via an anonymous online survey distributed in November 2017. A statistical significance level (p) was set at 0.05.

RESULTS: Over 3,300 cultures were sent during the 30 month study period: 1596 during the pre- and 1710 during the post-implementation periods of culture order indication documentation. There was a trend toward a decrease in overall number of cultures ordered per 100 patient days ($p=0.083$) and a decreasing trend toward the proportion of positive culture results after implementation ($p=0.125$). Notably, the proportion of clinically insignificant cultures decreased following culture order indication documentation ($p=0.015$). The two most common indications documented were temperature instability and other. Of 35 end user survey respondents, 22 (63%) indicated some level of difficulty with selecting an indication; although 32 (91%) stated that listing an indication did not impact efficiency. None of the respondents indicated that specifying an indication negatively impacted their practice.

CONCLUSION: Requiring an indication to be listed when ordering a blood, wound, or tracheal bacterial culture order among inpatients of a cardiac service line of a tertiary pediatric healthcare system led to fewer clinically insignificant positive culture results without impacting ordering efficiency.

13. Value-Driven Design of Pediatric Medical Devices

Bodner, Douglas

The medical device industry focuses primarily on the adult market. One issue is that device manufacturers believe that the pediatric market is too small to sustain profit for devices given the large research and development costs incurred. Another related issue is that generic adult devices are not easily adapted to pediatric use. In this research, we apply a value-driven design perspective to design of pediatric devices. In value-driven design, a fundamental goal is specified, often to maximize profit of the resulting device in uncertain environments through risk-adjusted utility. Profit is a function of demand and price, as well as production cost. Demand is a function of device attributes (i.e., what clinical benefits does it provide) and price, provided via a model of demand in the market. These attributes result from design decisions, and these decisions also drive the production cost in terms of fixed and variable cost components. The goal is to optimize the objective function over the set of design decisions and the price decision, noting that uncertainties are modeled when conducting the optimization, for example by Monte Carlo simulation.

This value-driven design framework has demonstrated benefits in regular consumer markets. However, the pediatric device market has a number of features that differentiate it from these market types. Four sources of differentiation include (i) insurance, which may offset the price to the consumer, (ii) greater risk to the manufacturer in terms of profit, (iii) subsidies available to offset

research and development costs, and (iv) fundamental objectives different from profit (e.g., health outcomes). We modify the value-driven framework to incorporate these issues and present generic models applied to pediatric device design. There are not standardized methods for health outcome evaluation in pediatric care. Thus, we initially consider quality-adjusted life years, as this metric fits with methods used to model risk preferences in value-driven design. Future research involves consideration of additional health outcome metrics and specific case study applications to demonstrate the value-driven approach.

14. A Microparticulate Approach to Influenza Vaccines Using Influenza Matrix Protein Virus-like Particles

Braz Gomes, Keegan; Braz Gomes, Kimberly; Kang, Sang-Moo; and D'Souza, Martin J.

INTRODUCTION: We investigated the efficacy and protectivity of a matrix-2 protein virus-like particle (M2e VLP) microparticulate (MP) vaccine administered transdermally in a pre-clinical influenza mouse model.

METHODS: The M2e VLP, and adjuvants MPL-A® and Alhydrogel®, were spray dried into polymeric microparticles. For in vivo testing, C57BL/6 mice were vaccinated with one prime and two booster doses intramuscularly (I.M.), or transdermally (T.D.) using a microneedle admin patch. Mice were later challenged with live influenza virus. Blood samples, lymphoid organs, and whole lung tissue was collected to analyze adaptive immunity using T cell lymphocytes and viral titers.

RESULTS: The M2e VLP, M2e VLP MP, and M2e VLP MP + MPL-A® + Alhydrogel® vaccine groups demonstrated elevated levels of IgG and IgG1, and high expression of CD4+ and CD8+ T cells. The lung viral titers were 10-fold lower in the M2e VLP MP + MPL-A® + Alhydrogel® vaccinated mice compared to M2e VLP and M2e VLP MP.

DISCUSSION: Since the current licensed vaccines against influenza are facing numerous challenges associated with production time, antigenic changes, and route of administration, we developed a flu vaccine with the M2e VLP that was easy to formulate, immunogenic, safe and protective.

CONCLUSION: Our transdermal influenza VLP vaccine using the conserved M2e protein could potentially serve as a feasible alternative to the currently available trivalent and quadrivalent intramuscular influenza vaccines.

15. Birth Delivery Mode Affects Development of the Perinatal Mouse Brain

Castillo-Ruiz, Alexandra; Mosley, Morgan; Jacobs, Andrew J.; Hoffiz, Yarely; Vasquez, Abigail; and Forger, Nancy G.

Birth entails profound changes in a newborn's environment and the processes associated with birth prepare key peripheral organs, such as the liver and lungs, for the transition to postnatal life. However, whether birth mode (vaginal vs Cesarean) influences the brain has received little attention. Given the high rates of Cesarean deliveries worldwide, it is crucial to understand how birth mode

can affect brain development. In this study, we investigated this question by focusing on two key developmental processes: cell death, which shapes neural connections, and colonization of the brain by microglia, the resident immune cells of the brain. We generated timed-pregnant C57BL/6 mice and collected the offspring in utero at embryonic day (E)18.5 and E19 and ex utero at postnatal day (P)0 (3h after birth), P1, and P3 following a vaginal or Cesarean birth. Brains were immunohistochemically stained for activated caspase-3, a cell death marker, and ionized calcium binding adaptor molecule 1, a microglial marker. We found that Cesarean born mice had a transient increase in cell death across many brain regions, including the paraventricular nucleus of the hypothalamus (PVN), which is of interest for its central role in the stress response and brain-immune interactions. We also found significant differences in microglial morphology in the PVN, with Cesarean born mice having a 20% reduction in soma size when compared to vaginally born mice at P1 and P3. Because soma size is a marker of microglial activation, our results suggest that microglia are less activated in Cesarean born mice. We are currently investigating whether these effects extend to other brain regions. To determine whether the effects of Cesarean delivery on cell death lead to lasting changes in cell numbers in the PVN, we collected the brains of vaginally and Cesarean born mice at P23. While we found no difference in total cell number, we did find that Cesarean born mice had fewer vasopressin producing cells. Together our results suggest that birth may be an important event for neurodevelopment and deviations from vaginal birth may alter brain development.

16. Plasma Neuregulin in Children with Sickle Cell Anemia

Chambliss, Christopher; Richardson, Tatayana; Onyekaba, John; Liu, Mingli; Buchanan-Perry, Iris; Darden, Trudie; Stiles, Jonathan; and Gee, Beatrice

BACKGROUND: Stroke is a common complication of sickle cell anemia (SCA, Hemoglobin SS). Children with SCA may have reduced cerebral blood flow as a result of chronic anemia, which can be further worsened in those with high stroke risk. We have previously found elevated plasma concentrations of brain-derived neurotrophic factor (BDNF) in children with SCA compared to healthy children, with the most elevated levels in those with high stroke risk [Hyacinth et al. *Cytokine* 2012 60(1): 302]. Brain-derived neurotrophic factor is a growth factor produced by ischemic brain. In the case of children with SCA and high stroke risk, it is likely to be a response to reduced cerebral perfusion. We are interested in finding additional biomarkers to help improve the accuracy of predicting stroke risk in children with SCA.

The focus of this study is Neuregulin-1 β (NRG-1), which has been found to be protective in ischemic stroke, cerebral malaria, and cardiomyopathy. NRG-1 is an endogenous polypeptide growth factor that triggers signal transductions through the actions of the ERB family of tyrosine kinases. Activation of this cascade has shown to be involved in nervous system development and recovery from injury. We hypothesize that NRG-1, being neuroprotective, will be increased in children with SCA due to reduced cerebral perfusion related to anemia. We measured NRG-1 in plasma from children with SCA, compared to healthy controls, using enzyme-linked immunosorbent assay (ELISA).

RESULTS: Plasma neuregulin was determined for 33 SCA subjects and 19 Controls. Subjects with SCA had significantly higher levels of NRG-1 when compared to healthy controls (median 2644 vs. 399 pg/ml, $p < 0.001$). Neuregulin levels for subjects with SCA and high stroke risk ($n=4$) were not different from the other SCA subjects.

CONCLUSIONS: Higher plasma concentrations of NRG-1 in children with SCA may reflect ongoing vascular injury and/or cerebral ischemia. In our small subset of children with SCA and high stroke risk, there was no significant difference in NRG-1 levels. Further studies with more children with high stroke will be needed to test the utility of neuregulin as a predictive biomarker for stroke risk.

17. Characterizing Late Effects Knowledge in Young Adult Survivors of Childhood Cancer

Chan, Melissa; Williamson, Rebecca; Gilleland Marchak, Jordan; Mertens, Ann; and Wasilewski-Masker, Karen

BACKGROUND: The growing population of childhood cancer survivors is at risk for therapy related late effects that persist or arise after treatment. Their own knowledge of specific exposure based risks impacts their engagement with medical professionals and pursuit of appropriate screening and intervention.

PROCEDURE: This cohort of 85 pediatric cancer survivors between the ages of 18-25 years old completed the Survivor Knowledge Questionnaire, reporting their risk for 11 exposure based risks. Their responses were compared with their treatment risks based on medical record review of exposures and as outlined in the COG LTFU Guidelines. Descriptive analysis and comparisons of mean total knowledge scores were made using two-way ANOVA.

RESULTS: The highest sensitivities were found across four late effects: fertility problems (78%), hearing problems (75%), heart problems (73%), and thyroid problems (70%). Mean knowledge scores were highest in Hispanics, higher household income, higher patient education level, and being at risk for a higher number of late effects.

CONCLUSIONS: There was increased ability to accurately identify risk when that risk was associated with screening or a visit with another subspecialty provider for screening (endocrinology; echocardiogram/cardiology; audiogram/audiology). Multidisciplinary education including through a subspecialty provider for a specific late effect may help increase the understanding of late effects risk in young adult survivors of childhood cancer.

18. Methionine Oxidation by Myeloperoxidase is Associated with Early Cystic Fibrosis Lung Disease

Chandler, Joshua D.; Margaroli, Camilla; Horati, Hamed; Kilgore, Matthew B.; Veltman, Mieke; Liu, H.

Ken; Scholte, Bob J.; Taurone, Alexander J.; Peng, Limin; Guglani, Lokesh; Uppal, Karan; Go, Young-Mi; Janssens, Hettie M.; Jones, Dean P.; and Tirouvanzi

RATIONAL: Cystic fibrosis (CF) lung disease progressively worsens from infancy into childhood and adulthood. Changes in CF airway fluid composition may reveal therapeutic targets to curb disease progression, especially in infancy.

AIM: Identify molecular drivers of lung disease in CF infants among airway fluid metabolites.

METHODS: CF patients (n=24) prospectively received bronchoalveolar lavage and chest computed tomography (CT) scan at age one or three years. Small molecules in BALF were measured with high-resolution, accurate-mass Orbitrap mass spectrometry (HR-MS). Myeloperoxidase was quantified by ELISA and activity assays. Lung disease was quantified from the chest CT scans by using the PRAGMA-CF score, which quantifies bronchiectasis, mucus plugging and other abnormalities, yielding a composite measure of structural damage (PRAGMA-%Dis). Spearman correlations were used for all data except untargeted metabolomics, in which data were log₂ transformed and quantile normalized so that Pearson's correlations were appropriate. Storey's q-value for multiple comparisons adjustment was used.

RESULTS: Increased PRAGMA-%Dis was primarily caused by bronchiectasis in this cohort, and was correlated with changes in BAL cell subsets. One-hundred and four m/z features from HR-MS were significantly correlated with PRAGMA-%Dis in a linear model ($p < 0.05$, $q < 0.25$). The top annotated significant feature was methionine sulfoxide (Pearson's $r = 0.648$, $p = 5.6 \times 10^{-5}$), a product of methionine oxidation by myeloperoxidase. We confirmed the identity of methionine sulfoxide, and quantified it in patient BALF using external reference calibration of untransformed mass spectrometry data, confirming a high correlation with PRAGMA-%Dis (Spearman's $\rho = 0.582$, $p = 2.9 \times 10^{-3}$) and bronchiectasis (PRAGMA-%Bx; $\rho = 0.698$, $p = 1.5 \times 10^{-4}$). Myeloperoxidase levels also correlated with PRAGMA-%Dis ($\rho = 0.706$, $p = 1.7 \times 10^{-4}$), PRAGMA-%Bx ($\rho = 0.752$, $p = 3.5 \times 10^{-5}$) and methionine sulfoxide ($\rho = 0.803$, $p = 3.9 \times 10^{-6}$).

CONCLUSIONS: BALF methionine sulfoxide correlates with structural airway damage and myeloperoxidase levels in CF infants. Further studies are needed to establish methionine oxidation as a mechanistic biomarker and therapeutic target relevant to early CF airway disease.

19. Evolution of Pediatric Inflammatory Bowel Disease Unclassified (IBD-U): Incorporated with Serological and Gene Expression Profiles

Chandradevan, Raguraj; Tatyana, Hofmekler; Mondal, Kajari; Venkateswaran, Suresh; Somineni, Hari; Ballengee, Cortney; Sauer, Cary; Hyams, Jeffrey; and Kugathasan, Subra

BACKGROUND: Inflammatory bowel disease (IBD) mainly consists of Crohn's disease (CD) and ulcerative colitis (UC). About 10-15% of patients with IBD cannot be firmly diagnosed with CD or UC, hence they are initially diagnosed as inflammatory bowel disease unclassified (IBD-U). Having

a firm diagnosis is clearly preferred to guide treatment choices, and better understanding of the nature of IBD-U is required.

METHODS: We performed an analysis of a subset of pediatric subjects from an inception IBD cohort of patients initially enrolled in a prospective multi-center study (the RISK study). Initial diagnosis and 2 year-follow-up data from the subjects diagnosed with IBD-U were analyzed. An expert panel verified final diagnosis using pre-defined criteria as a guide. Serological and disease-relevant ileal and rectal tissue gene expression profiles were investigated. The use and the time to initiate anti-TNF- α treatment was analyzed among the outcome groups.

RESULTS: A total of 1411 subjects were enrolled with initial diagnosis of IBD and among them 136 subjects were initially diagnosed as IBD-U at enrollment and 26% were reclassified as UC and 14% as CD within 2 years of diagnosis, while 60% remained as IBD-U. Of those who were reclassified, there was a 2:1 ratio, UC (n=35) to CD (n=19). The molecular and serological features of IBD-U at the end of follow-up were very similar to UC and very different from CD. There was less likelihood of receiving anti-TNF- α agents if the diagnosis was IBD-U compared to CD (P<0.0001).

CONCLUSIONS: In our cohort, 60% of the initial IBD-U subjects remained as unclassified at 2 years and of those subsequently classified a higher (26%) turn out to be UC. Most of the IBD-U subjects had serological and molecular signatures at final diagnosis that appear very similar to UC. Despite the atypical features led the initial diagnosis of IBD-U, molecular and serological factors support they were more likely to be UC than CD. However, long-term studies are needed to better understand the natural history and molecular characterization of pediatric onset IBD-U.

20. Development of Autoantibodies During Pemphigus Vulgaris Pathogenesis

Cho, Alice; Caldara, Amber; Ran, Nina; Payne, Aimee; Kowalczyk, Andrew; Feldman, Ron; and Wrammert, Jens

Pemphigus vulgaris (PV) is a B cell-mediated autoimmune disorder that causes widespread blistering on skin and mucosal membranes. The autoantigenic target of PV is a cellular adhesion molecule in the skin called desmoglein-3 (Dsg3). However, little is known about the origin of Dsg3-specific B cells or how they develop to cause disease.

In our study, we are characterizing disease pathogenesis at a single-cell level by generating a panel of Dsg3-specific monoclonal antibodies (mAbs) from patient memory B cells (MBCs) isolated at the time of diagnosis. We found that both an increase in anti-Dsg3 serological titers and the emergence of Dsg3-specific MBCs correlated with presentation of symptoms in patients. Using a recombinant Dsg3 probe, we used flow cytometry to detect and single-cell sort Dsg3-specific MBCs. Interestingly, these cells exhibited an activated phenotype, suggesting that these MBCs are part of ongoing immune responses. We next used multiplex PCR to generate mAbs derived from these single cells. Immunoglobulin repertoire analysis showed that the mAbs were highly clonal, implying that a small pool of clonally restricted B cells dominates the autoimmune response. MBCs were highly mutated, suggesting ongoing antigen-driven affinity maturation. While mAbs were specific for Dsg3, with no

binding towards irrelevant influenza and cholera proteins, they had a wide range of affinities for Dsg3. When testing these antibodies for in vitro pathogenicity, it was clear that Dsg3 affinity of mAbs did not directly correlate with the potency of pathogenicity

Overall, Dsg3-specific mAbs are antigenically selected and oligoclonal, with a range of functional properties. Future experiments to define epitope specificity and differences between pathogenic and non-pathogenic mAbs will help us understand the role of MBCs in PV pathogenesis, and contribute to our current understanding on the development of autoimmune B cells.

21. Age, Height, Weight, and MRI Measurements Are Not Reliable Predictors of Hamstring Graft Size in Adolescent Patients

Corey, Sally; Perkins, Crystal; Braithwaite, Kiery; Rostad, Bradley; Willimon, S. Clifton; Busch, Michael; and Christino, Melissa

BACKGROUND: The semitendinosus and gracilis tendons are commonly used for anterior cruciate ligament reconstruction (ACLR) in adolescent athletes. The size of these tendons varies between individuals, and smaller hamstring graft size has been associated with the risk of graft failure. The purpose of this study was to determine if age, height, weight, BMI, and MRI measurements of hamstring tendon cross-sectional area can predict intra-operative hamstring graft size.

METHODS: This IRB-approved, retrospective review was performed on patients who underwent ACLR using quadrupled hamstring autograft from August 2012-December 2015. Intra-operative hamstring graft size was compared to height, weight, body mass index (BMI), age, and MRI hamstring measurements using a method described by Bickel, et al for adolescents. MRI measurements were performed and repeated by three different observers using the freeform region of interest tool and recorded as the cross-sectional area (CSA) of both tendons combined. Inter- and intra-rater reliability were analyzed with intra-class coefficients. Correlation was described with Pearson correlation coefficients and regression models.

RESULTS: 256 patients with an average age of 15.1 years (range 10 - 19 years) were included. Females comprised 54% of patients. The average graft diameter was 7.96 mm (range 5-10 mm). Age, weight, BMI, and height all had low Pearson correlation coefficients at $r=-0.07$, 0.19 , 0.06 , and 0.3 respectively, $p=0.19$, 0.002 , 0.001 , and 0.001 . The inter-rater reliability for MRI measurements of CSA using the Bickel method was $ICC=0.54$. The intra-rater reliability was $ICC=0.80$.

CONCLUSIONS: Anthropometric measurements, including age, height, weight, and BMI are not useful predictors of intra-operative hamstring graft size in adolescents. There is a correlation between hamstring graft size and the Bickel method of hamstring CSA, but it is not a clinically reliable measurement. The ability to reliably anticipate intraoperative hamstring graft size would be a valuable aspect of surgical planning, however further study is needed to identify optimal factors that will predict this in young patients.

22. Barriers to Families Receiving Diagnoses and Services After a Positive Autism Screen in a Community Screening Study

Costo, Megan; Kaiser, Eileen; and Stapel-Wax, Jennifer

1 in 68 children are identified with Autism Spectrum Disorder (ASD). The earliest signs of ASD are delays in social communication milestones that appear in the first 2 years, yet most children are not diagnosed until 4-5 years of age. Underserved families are identified even later and significantly underrepresented in research. The American Academy of Pediatrics recommends screening all children for ASD at 18 and 24 months. The community faces many barriers to early screening and detection, including limited uptake of ASD screeners in pediatric practices and conflicts with the realities of early intervention services. Sometimes professionals prefer to wait and see, due to uncertainties in subtle symptom-expression early on and the stability of an early diagnosis, though research shows that at age two, diagnoses are generally reliable and stable (Zwaigenbaum et al., 2009).

Even if children are screening for autism at 18 months, there are other significant barriers to children getting a diagnosis and receiving services, including parental ambivalence and the ability to act on referral recommendations, the capacity of professionals to recognize early red flags and make a diagnosis of ASD at a young age, and the knowledge and skills of EI providers in Part C to recognize ASD in toddlers and provide evidence-based early interventions. In this multi-site community screening study, primary care practices, early learning centers and members of faith-based organizations were recruited to become community screeners. Community screeners received training to recognize early signs of autism, share screening results, and provide next steps to families. After a positive screening result, families were encouraged to seek early intervention services and were offered a no-cost diagnostic evaluation.

Currently 2,041 families have taken the screener. Of the 95 families whose child had a positive outcome on the screening, 35 completed the evaluation, 28 were not interested, 25 could not be reached, and 7 were cancellations or no-shows that could not be reached to reschedule. We will explore further some of the barriers to families completing a free evaluation and getting into early intervention services in this poster as well as family demographics and qualitative feedback.

23. The Influence of Maternal Immunity on Infant BCG Vaccine Response

Cranmer, Lisa; Jaspán, Heather; Sasser, Loren; Kagina, Ben; Gillespie, Scott; Njuguna, Irene; Nduba, Videlis; Wamalwa, Dalton; John-Stewart, Grace; and Day, Cheryl

BACKGROUND: The maternal immune system is critical in shaping early infant immune responses through transplacental transfer of antibodies. However, the influence of maternal antibodies to mycobacteria on the neonatal immune response to Bacille Calmette Guerin (BCG) vaccine against *Mycobacterium tuberculosis* (Mtb) has been relatively unexplored.

METHODS: In a cohort of Kenyan mother-infant pairs, we measured IgG to purified protein derivative (PPD), lipoarabinomannan (LAM), and *M. tuberculosis* whole cell lysate (WCL) in

maternal and infant blood at delivery, and in maternal breast milk at 6 weeks postpartum. We assessed infant BCG vaccine immunogenicity at 10 weeks post BCG vaccine using a whole blood cell assay to measure the frequency of BCG-specific Th-1 cytokine+ CD4 cells. We evaluated the association of maternal and infant humoral immune responses to mycobacterial antigens in blood and breast milk with infant BCG immunogenicity using univariable and multivariable linear regression.

RESULTS: Among 56 Kenyan mothers, anti-mycobacterial antibodies were detectable in plasma and breast milk. The efficiency of transplacental transfer was similar for all mycobacterial antigens, with median cord-to-maternal ratios of 0.59 (PPD), 0.68 (WCL), and 0.69 (LAM). On univariable linear regression, maternal anti-PPD IgG and anti-WCL in blood and anti-PPD IgG in breast milk were associated with decreased infant BCG immunogenicity. After adjusting for infant birth weight, maternal anti-WCL IgG in blood was independently associated with decreased infant BCG immunogenicity ($p=0.003$).

CONCLUSIONS: Maternal anti-WCL IgG in blood diminished infant BCG immunogenicity. Future studies to evaluate the impact of delayed infant BCG vaccination on efficacy of BCG to prevent Mtb in infants are warranted.

24. CD28 and CTLA-4 Requirements of Primary CD8+ T cell Responses to a Murine EBV Homolog

Crepeau, Rebecca L; and Ford, Mandy L

Treatment with CTLA-4 Ig blocks the shared ligands for CD28 and CTLA-4, CD80/CD86, thus blocking both CD28-mediated costimulation and CTLA-4-mediated coinhibitory signals that serve to dampen effector T cell responses and promote Treg-mediated suppression. Treatment with belatacept, a CTLA-4 Ig fusion protein, is contraindicated for patients that are EBV negative, due to a ten-fold increase in the rate of virally-induced post-transplant lymphoproliferative disease (PTLD). PTLD has long been considered a result of dysregulated and impotent virus-specific CD8+ T cell responses generated in the absence of CD28 costimulation. Recently, anti-CD28 domain antibodies that selectively target CD28 while leaving CTLA-4 intact have been developed, providing a promising new avenue for immunotherapy for autoimmunity and transplantation. Here, we sought to determine whether the potent immunosuppressive effects of selective CD28 blockade treatment would result in diminished anti-viral protective immunity compared to CTLA-4 Ig. To address this, we infected mice with a YFP labeled recombinant MHV-68, a murine model of EBV. Following infection and treatment with either PBS, CTLA-4Ig, or anti-CD28 dAb, we monitored the kinetics of virus-specific CD8+ T cell expansion and differentiation, as well as viral load, in the blood. Preliminary results revealed a decrease in p79-tetramer positive CD8+ T cell frequencies in both treated groups as compared to untreated animals. However, there was also a further decrease in frequencies of antigen-specific CD8+ T cells in animals treated with the anti-CD28 dAb as compared to CTLA-4 Ig. We also observed a concomitant increase in the proportion of virally infected cells in the blood at day 7 post-infection in those mice treated with anti-CD28 dAb as

compared to CTLA-4 Ig. These early results suggest that blockade of CTLA-4 mediated coinhibitory signals in the context of CTLA-4 Ig may serve to mitigate suppression of viral-specific CD8+ T cells occurring as a result of impaired CD28 signaling. Additionally, selective CD28 blockade, which preserves physiologic CTLA-4 mediated coinhibition, may result in increased suppression and dysregulation of virus-specific CD8+ T cell responses following MHV infection.

25. Thyroid Disruption In People Exposed To Polybrominated Biphenyl (PBB) As Children

Curtis, Sarah W.; Terrell, Metrecia L.; Marcus, Michele; Conneely, Karen N.; Smith, Alicia K.

During the 1970's, Michigan residents were exposed to polybrominated biphenyl (PBB), an endocrine disruptor, during an agricultural accident. Children were exposed during this incident by directly eating contaminated food products or through breastfeeding, or were exposed in utero through placental passage. Studies in rat models show that PBBs can cause altered thyroid hormone levels, and that the effects of PBBs can vary based on age of exposure. Epidemiological studies show that people who were exposed before puberty report numerous developmental and endocrine-related health problems, even though those exposed as children have significantly less PBB exposure than those exposed as adults (2.12 ppb vs. 5.90 ppb; $p=9.0e-09$). In order to investigate whether PBB exposure during critical developmental time-points was associated with an increased risk for health problems, we evaluated 387 registry participants exposed before the finished puberty. Age acceleration, operationalized as the difference between chronological age and a DNA methylation-based estimate from 353 CpG sites, associated with increased PBB levels ($p=0.04$), when adjusted for age, gender, and cell type estimates. This supports the hypothesis that children exposed to higher levels of PBB may be at increased risk for adverse health outcomes. Examination of health questionnaire data revealed that 21% of those exposed to PBB before puberty reported having thyroid dysfunction, compared to only 7% of those exposed as adults ($p=8.2e-05$). Next, free T3 and free T4 were measured in serum samples from participants who were not taking thyroid medication (N=344), using a chemiluminescent immunoassay analyzer. There was a significant, positive association between PBB exposure level and free T3 levels ($p=0.016$), after controlling for age and gender. Together, this indicates that people who are exposed to environmental contaminants as children may have an increased risk for endocrine-related health problems, like thyroid disruption, that persist throughout their lives.

26. Cytoprotection of Intestinal Tissue by *Lactococcus lactis*

Darby, Trevor; Owens, Joshua; Saeedi, Bejan; Luo, Liping; and Rheinallt M. Jones

The use of beneficial bacteria known as probiotics, defined as "live microorganisms which when consumed in adequate amounts, confer a health benefit to the host," is widely practiced. Nevertheless, experimental evidence corroborating the efficacy of many bacteria promoted with such claims remains inadequate. We directly address this gap by identifying a novel beneficial bacterium

that potently dampens intestinal inflammation and limits tissue damage. Utilizing both a *Drosophila* & murine animal model, we identify a *Lactococcus lactis* species that potently defends the fly gut from induced oxidative damage, as well as eliciting cytoprotective activity against radiological damage. Importantly, the beneficial activities of this strain of *Lactococcus* in the murine intestine were significantly stronger by comparison to other currently marketed beneficial bacteria. In summary, we demonstrate that capacity of a beneficial bacteria to elicit potent anti-inflammatory and cytoprotective properties in the gut and propose that newly discovered probiotic strains of bacteria may be used as a therapeutic intervention to promote intestinal health.

27. The Utility of Cardiac Exercise Stress Testing in the Evaluation of Pediatric Patients with Chest Pain

Dasgupta, Soham; Stark, Megan; Bhatt, Sonal; and Deshpande, Shriprasad

BACKGROUND: Chest pain is a frequent symptom in pediatric patients with cardiac causes being extremely rare. Despite its low sensitivity/specificity, exercise testing has remained one of the widely used noninvasive tests to determine the prognosis in patients with suspected coronary disease. We aimed to look at the utility of cardiac exercise stress testing in the evaluation of pediatric patients with chest pain.

METHODS: After obtaining institutional review board approval, we performed a retrospective chart review of all inpatient and outpatient referrals for an exercise stress test for symptoms of chest pain between January 2014-2017. Patients with prior exercise stress tests were excluded.

RESULTS: A total of 389 patients met inclusion criteria. Fifty-seven of them had known congenital heart disease. Echocardiogram (ECHO) was performed on 333 (85.6%) patients and 43 (11%) previously unknown structural cardiac anomalies were identified. The 3 most common anomalies identified were patent foramen ovale [n=6], bicuspid aortic valve (BAV) [n=6] and left ventricular hypertrophy (LVH) [n=5]. A total of 79 (20.3%) patients had an abnormal exercise stress test with the 3 most common causes all being related to the respiratory system (obstructive pattern on spirometry [n=34], exercise induced asthma [n=22] and restrictive pattern on spirometry [n=6]). There were only 5 patients who had both an abnormal exercise stress test and an incidental structural anomaly on ECHO - two patients with ST elevations during stress testing had LVH on ECHO, 1 patient with a blunted blood pressure response to exercise had a BAV while the other 2 patients had valvar pulmonary stenosis and an aberrant right subclavian artery but only pulmonary anomalies on the stress test.

CONCLUSION: A very low percentage of patients previously undiagnosed with heart disease had an abnormal stress test and an incidental anomaly on ECHO (1.5%). This reiterates the fact that chest pain in the pediatric population is rarely cardiac in origin. Furthermore, the majority of abnormal stress tests were secondary to a pulmonary cause which may explain the chest pain. This emphasizes the need of performing a complete cardio-pulmonary stress test including pulmonary function tests in the evaluation of patients with chest pain.

28. Medulloblastoma Organotypic Slice Cultures: From Deciphering Tumor Microenvironment To Delivering Precision Medicine

Dey, Abhinav; Malhotra, Anshu; Felker, James; Liu, Jingbo; Scheniderjaan, Matt; Ahn, Song Ih; Hovell, Candice; Sei, Yoshitaka; Virtue, Theodore; Kim, Yongtae; MacDonald, Tobey; and Kenney, Anna

Medulloblastoma (MB) is the most common solid malignant tumor found in children. These tumors arise in young children in the cerebellum, a part of the brain that develops postnatally in humans and in mice. Using mouse models for the Sonic hedgehog (SHH) subgroup of MB, we have discovered a novel signaling network comprising SHH, Yes-Associated Protein-1 (YAP), Y-box Binding protein-1 (YB1) and Insulin-like Growth Factor-2 (IGF2) that promotes proliferation of tumor cells. In our recent Oncogene publication (2016) (1), we showed that knockdown of YB1 in postnatal day 7 cerebellar organotypic slice cultures (OSC) reduced the thickness of external granule layer (EGL), where proliferating cerebellar progenitor cells reside, emphasizing that the proliferative effect of YB1 is maintained in a cerebellar microenvironment. OSC, thus, preserves several important architectural features of the host tissue, such as neuronal connectivity, cellular stoichiometry, and tumor-stroma interactions.

Development of more effective, less harmful therapies has been slow due to a poor understanding of molecular mechanisms underlying therapy resistance. We have utilized the OSC method to investigate mechanisms of radiation-resistance adopted by YB1 in the murine MB tumor microenvironment (TME) known as the peri-vascular niche (PVN), which harbors tumor re-populating cells. Subsequently, to screen for novel therapeutics and efficient nanoparticle-based drug-delivery systems that successfully maneuver the TME, we employed the OSC technique to determine the feasibility of targeting drivers of radiation resistance found in the PVN.

We will also show evidence of the utility of OSC in testing inhibitors on MB patient-derived tissue, illustrating the applicability of OSC for patient-tailored therapy. We have also designed a novel, high throughput, microfluidic device that integrates the OSC with a microfluidic chemical gradient generator for preclinical combination drug delivery studies. We hypothesize that this device will provide rapid and reliable prediction of the best possible drug combinations for precision medicine.

29. Perceptions of Transition from Pediatric to Adult Care for Youth with Chronic and Rare Diseases: A Participatory Health Research Project

El-Sayegh, Lydia; Smith, Sharon; Thompson, Charlie; and Tsang, Vivian W. L.

BACKGROUND: Children with chronic and rare disease have challenges in transition into adult care.

OBJECTIVE: To understand physician perceptions of current protocols for the transition of pediatric patients with a history of childhood disease into adult care and expand future research to include the perceptions of youth.

METHODS: 1) A survey to investigate physician perception on existing gaps in the transition of care in children's hospitals around the world and to identify the barriers and improvements that can be made was administered by youth to 100 physicians at the 2017 American Academy of Pediatric National Conference & Exhibition. 2) 15 semi-structured, standardized interviews of medical professionals who work with adolescents were created and administered by youth to investigate adolescent transitions in 8 cities across Canada and the United States.

RESULTS: In Phase 1, 65% of respondents were medical providers (MD, DO), 17.17% were students/residents/fellows, and 6.06% were researchers (PhD, MS/MA). 57% rated current processes of transitioning from pediatric to adult care mean 5 out of 10 on a 10 item scale with no participants rating 9 or 10. Top obstacle was a lack of communication between pediatric and adult doctors (68%). The top ranked strategy for improvement was to provide formal transition guidelines (68%). In Phase 2, 66.67% of respondents were pediatricians, with 60% of interviewees were in public healthcare and 40% were in private healthcare. There are evident unmet needs in the transition process as identified by healthcare personnel. Creation of general transition guidelines or formalized transition models (66.67%), tools to navigate insurance processes (66.67%), and increased access to adult specialists interested in adolescent medicine (46.67%), were reported to be among the most needed additions to supplement the transition process.

CONCLUSION: Continued assessment and research are necessary to establish trends in pediatric hospitals internationally, to share best practices on this issue. Currently, ongoing work is taking place to interview youth at the same pediatric institutions to ask them how models of transition can be improved.

30. Investigating the Role of Lysine Specific Demethylase 1 (Lsd1) in Retinal Development and Retinoblastoma Differentiation

Ferdous, Salma; Grossniklaus, Hans E.; and Nickerson, John M.

PURPOSE: The purpose of this study was to determine the role of lysine specific demethylase 1 (Lsd1) in retinal cell differentiation. Lsd1 specifically removes H3K4 and H3K9 methylation. Popova et. al found that late progenitor retinal cells express Lsd1 as they become postmitotic and begin to differentiate. Proper retinal differentiation is important for normal visual function, but also aberrantly occurs in retinoblastoma, the most common primary pediatric intraocular tumor. These tumors display several hallmarks features, namely, Homer Wright (HW) and Flexner - Wintersteiner (FW) rosettes, which mimic retinal differentiation, and fleurettes, which mimic photoreceptor differentiation. Because rosettes and fleurettes mimic general retinal differentiation, we investigated the role of Lsd1 in normal murine retinal development.

METHODS: Retinoblastoma affected eyes were enucleated, fixed with paraformaldehyde, and underwent immunohistochemistry for Lsd1. Additionally, immunohistochemistry was conducted on murine retinal sections at various stages during and after development.

RESULTS: In retinoblastoma tumors, Lsd1 shows remarkable expression in highly differentiated areas, but is absent in undifferentiated areas. Murine retinal sections show high expression of Lsd1 in all retinal progenitor cells during development, but cell-type specific expression after development. In mature retinas, Lsd1 is expressed in all three nuclear layers, the retinal ganglion cell layer (RGC), inner nuclear layer (INL), and outer nuclear layer (ONL). The INL shows uniform staining, however, the RGC and ONL show variation. Co-labeling of Lsd1 with short-wavelength cone opsin pigment (S-OPSIN) reveal that Lsd1 expressing cells in the ONL are cone photoreceptors.

CONCLUSIONS: These experiments highlight Lsd1 involvement in the differentiation of particular retinal cell subtypes and possible contribution to the differentiation and aggression of retinoblastoma tumors. Currently, Lsd1 inhibitors are in clinical trials for the treatment of various cancers, including acute myeloid leukemia (AML) and lung cancer. Therefore, we hypothesize that these inhibitors may be a potential therapeutic strategy for retinoblastoma.

31. Camp Influences on Physical Activity in Pediatric Oncology Patients and Survivors

Fialkowski, Allison; Thomas, Jasmine; Huang, Xiqin; Jordan, Dorothy; and Withycombe, Janice

In the USA and Canada, over 85 summer camps serve pediatric cancer patients. However, little research has assessed the physical benefits of camp attendance, especially in camps for children with illnesses. Therefore, this work analyzes camp's effect on levels of physical activity in pediatric oncology patients and survivors. Further, these analyses examine associations between physical activity, gender and Body Mass Index (BMI). As physical inactivity is hypothesized to contribute to poor overall health, interventions to support physical activity have potentially profound utility to improve quality of life in pediatric oncology patients and survivors.

During June 2017, thirty pediatric oncology patients and survivors (8-17 years of age) were recruited from a convenience sample of registered campers attending a 6 day residential oncology camp. Sample demographics include: 57% male, 70% Caucasian, 20% Asian, 7% African American, and 1% Hispanic; 46%, overweight (n=7) or obese (n=7). Of these 30 participants, 27 completed the study and were included in data analysis. In addition to standard demographic data, height and weight measurements were obtained to calculate BMI. Physical activity was assessed using a Garmin Vivofit monitor for one week before camp as well as during camp to objectively measure steps/day.

The children significantly increased their physical activity at camp by approximately 7709.9 steps/day on average, $p < 0.001$, 95% CI [6209.2, 9210.1]. Mean steps do not significantly differ by gender during pre-camp week, $p = 0.70$, 95% CI [-2027.2, 2944.5], but boys did have significantly more steps during camp $p = 0.010$, 95% CI [-5818.0, -844.1]. Additionally, before camp, the underweight/normal BMI campers have significantly more steps per day than overweight/obese campers, $p = 0.012$, 95% CI [712.5, 5253.7]; however, at camp, there is no significant difference in steps between the BMI groups, $p = 0.653$, 95% CI [1475.1, 14141.6].

These results support camp as a creative way to promote physical activity in pediatric oncology patients and survivors. As current studies support physical activity to reduce fatigue and other late effects in adult oncology patients and survivors, an intervention to promote physical activity could significantly benefit this population. The Oncology Nursing Society Foundation funded this study.

32. Reconstitution of Peripheral Blood Following Bone Marrow Transplantation in Sick Kids

Finlayson, Michael; Medrano-Trochez, Camila; Gibson, Greg; and Stenger, Elizabeth

BACKGROUND: Following bone marrow transplantation (BMT), the immune system is reconstituted as a mixture of derivatives of residual patient stem cells, and donor stem cells. The dynamics of this process must influence how the patient tolerates the graft without rejection, while also impacting to what extent the activity of the new mature immune system more resembles the host or donor. We used transcriptomics to follow the reconstitution of white blood cells in six bone marrow transplantation patients over two months.

METHODS: RNA was extracted from whole peripheral blood stored in Tempus tubes, sampled immediately prior to BMT and at days 7, 14, 21, 28, 56, and 90 from six patients as well as their matched donors. RNA-Seq was performed by single-end 100bp Illumina sequencing, reads were aligned to the reference human genome GRCh38 with STAR aligner, counted with HTSeq, and normalized with respect to library size using DESeq2. A novel clustering method was developed to monitor the emergence of overall transcriptomic profiles over time and to match host and donor profiles. Genotypes were called with GATK and comparison of the frequencies of homozygous polymorphisms between pairs of samples was used to estimate the proportions of cells in the major leukocyte types deriving from each donor.

RESULTS: Over the 8 weeks of the experiment, all six donors showed a gradual transition of transcriptomes representing reconstitution of the immune system. No clear pattern of gene expression more closely resembling host or donor was observed, likely because maturation was only partially complete. Genotyping of RNA shows temporal increases in donor contributions that follow different trajectories for particular genes that are likely to track with the emergence of each of the immune cell types.

CONCLUSIONS: RNA-Seq provides a novel approach to following immune maturation following BMT, with implications for understanding graft versus host disease and other conditions that afflict children recovering from life-threatening blood disorders.

33. Collaborative Developmental Monitoring to Provide Optimal Individualized Services for Children with Cerebral Palsy: the On Track Developmental Monitoring System

Fiss, Alyssa; and Jeffries, Lynn

Cerebral palsy (CP) is the most common motor disorder diagnosed early in life. Children with CP present with a complex mixture of health impairments and limitations with functional movement that develop at variable rates through childhood. Families with children with CP are diverse and health care systems vary. Health care providers collaborate with families to make decisions about the focus of episodes of care to improve functional movement and participation in life activities as children develop and age. While information on the clinical course of gross motor development for children with CP has been available using the Motor Growth Curves (Rosenbaum et al., 2002), there has been a gap in fundamental knowledge of the clinical course of development of impairments and participation of children with CP, both of which are important priorities for families with children with CP. The “On Track” study collected longitudinal data from 708 children with cerebral palsy (CP), age 1.5-11.9 years, to create the On Track developmental monitoring system (DMS). The On Track DMS consists of longitudinal trajectories and percentile graphs of the development of balance, range of motion, strength, endurance, walking and physical activity, health, participation in family/recreation activities, and performance of self-care activities. These data represent the current clinical course of young children with CP, previously not available. These data can be used within collaborative conversations between health care providers and families with children with CP to discuss realistic prognoses and to track and monitor development over time as compared to children with CP with similar functional ability (Gross Motor Function Classification System). Use of the On Track DMS should facilitate health care providers and families to more appropriately focus interventions on impairments and activities in which children are doing less than expected compared to peers, thus improve efficiency of services. This presentation will introduce the On Track DMS, provide examples of the longitudinal developmental trajectories and percentile graphs available in the On Track DMS, and highlight examples of how to use the system clinically to inform practice.

34. Calcineurin-dependent Immune Evasion During Leukemogenesis Mediated by a Novel, Targetable Protein, S15

Fonseca, Jairo A; Dougan, Jodi; Gardner, Lori; Rabe, Jennifer; Gearheart, Christy; Henry, Curtis; and Porter, Christopher C.

Acute Lymphoblastic Leukemia (ALL) is the most common pediatric cancer and the most frequent cause of death from cancer under age 20 in the US. BCR-ABL, a constitutively activated tyrosine kinase is present in 3-5% of pediatric ALL and is associated with poor prognosis. Development of tyrosine kinase inhibitors has significantly improved the treatment outcomes for BCR-ABL1+ALL patients, but these patients remain at high risk for relapse as the disease is not entirely eradicated by tyrosine kinase inhibition, making the development of novel therapeutics for BCR-ABL1+ALL a priority.

Previous studies by our group in a clinically relevant murine model of BCR-ABL1+ALL, have shown that leukemic cells deficient in calcineurin (a molecule related with lymphocyte development) are recognized by the immune system since the transplant of calcineurin-deficient cells in immunocompetent mice engraft in the bone marrow but are rapidly suppressed to undetectable levels. Furthermore, this effect is lost after pharmacological or genetic immunosuppression.

Transcriptome profiling of calcineurin deficient ALL revealed lower levels of a molecule called S15 when compared with control ALL. In addition, we observed a higher expression of S15 in human B lymphoblasts in comparison with normal lymphocytes.

Here we aim to elucidate the role of S15 in leukemogenesis and immune evasion through the knocking-down of S15 in murine BCR-ABL1+ALL. S15 knockdown was confirmed by a significant reduction in S15 mRNA levels and S15 surface expression. Transplantation of S15 deficient ALL in immunocompetent mice resulted in a slower rate of progression, lower leukemic burdens, and higher survival when compared to in control ALL cells. We assessed the therapeutic potential of interventions targeting S15 by using an anti-S15 monoclonal antibody and observed a reduction of leukemia incidence and leukemic burden.

Immunophenotyping of lymph node dendritic cells revealed higher levels of PDL2 in mice transplanted with control ALL. PDL2 negatively regulates T cells responses by inducing immune tolerance, suggesting that S15 can mediate immune evasion.

Our results suggest that S15 is a molecule related to leukemia immune evasion and that its targeting via monoclonal antibodies represents a promising therapeutic for the treatment of BCR-ABL1+ALL.

35. Divergent Patterns of Time-Varying Visual Attention to Social Stimuli in Toddlers with Autism Spectrum Disorder and Williams Syndrome

Ford, Aiden; Markert, Sarah; Olmstead, Jack; Klin, Ami; Shultz, Sarah; Lense, Miriam; and Jones, Warren

When looking at the world, a child faces an array of nearly unlimited visual information, yet at each moment in time she can look at just one thing. Remarkably, the visual scanning patterns of typically developing (TD) toddlers are synchronized: the majority of TD toddlers look to the same stimuli at the same moments in time, collectively orienting in a tightly time-locked fashion that scaffolds their social learning. Disruptions to these instantaneous spatial and temporal distributions of visual attention alter how and when children imbue meaning to signals in their environment, potentially driving developmental cascades toward atypical processing of the social world. In this analysis we quantify moment-by-moment patterns of visual scanning in toddler cohorts of children with autism spectrum disorder (ASD) and Williams Syndrome (WS), neurodevelopmental disorders with distinctive profiles of social disability. Eye-tracking data were collected from ASD and WS cohorts (mean age 33 months), as well as chronological and mental-age matched TD controls, while they viewed clips of children engaged in naturalistic social interactions. Time-varying kernel density estimation modeled each child's gaze as a continuous distribution function, permitting for analysis of both individual and group behaviors. Across moments when cohorts were synchronized (looking at the same location at the same time to a degree greater than predicted by chance, $p < 0.05$), children with WS most often converged on facial stimuli, in contrast to children with ASD who preferentially oriented to body and object stimuli. Additionally, the visual scanning of children with ASD and WS significantly differed from 18.31% and 3.75% of moments of TD visual synchrony, with little overlap

in group-specific moments of divergence. Results show that children with ASD and WS curate distinct visual environments and experiences and do not find meaning in the same stimuli at the same moments as their TD counterparts nor as each other. Ultimately, these analyses reveal altered topographies of salience in WS and ASD and offer insight into how atypical allocation of attentional resources in toddlerhood may contribute to the emergence of maladaptive social phenotypes in WS and ASD.

36. Design and Evaluation of the Usability and Utility of an MRI Training System for Children with Autism Spectrum Disorder

Foster, Amanda; Bartlett, Chris M.; Baranak, Andrew; Fain, Brad; Shultz, Sarah; and Denham, Megan E.

BACKGROUND: MRI-associated anxieties are magnified for children with Autism Spectrum Disorders (ASD) because of increased sensitivity to stimulation. This, along with intellectual disabilities, can result in more movement and agitation during the scan. When a child with ASD requires an MRI, the child is commonly sedated to guarantee the acquisition of high-quality data. There are, however, inherent risks associated with sedation. The Marcus Autism Center uses an MRI simulator coupled with a behavioral training protocol to successfully scan children with ASD while awake. Complicating this approach for children with ASD is their difficulty generalizing experiences from one situation to the next. This translates to a jarring experience shift when participants move to the actual scanner, even if they are comfortable in the simulator environment.

METHODS: The primary goal of this project was to create consistent, less intimidating simulated and actual MRI experiences for children with ASD, resulting in increased success rates for scanning without sedation and a reduction in the number of simulated sessions needed for acclimation. Researchers conducted collaborative design activities to solve the shift from simulator to MRI. Stakeholder feedback narrowed ideas to the most equitable few. Three iterative design cycles occurred to finalize the designs.

RESULTS: Modifications were made to the MRI simulator to resolve visual differences between the simulator and the actual MRI. After the bed height and head coil mounts were corrected, a foam representation of the MRI scanner was mounted to the simulator. Having a sense of control was the most insightful finding during interviews with families. To address this, an ambient lighting system was installed to allow the children to choose the color of the room. A cloth curtain hung across both rooms tangent to the scanners obscured distractions behind the scanners.

CONCLUSION: This research executed a human-centered design methodology to implement a solution that potentially reduces the use of sedation for children receiving MRIs. The use of an MRI simulator and modifications to the environment increase child comfort and likelihood for successful data collection. The modifications are expected to improve the user experience for children with ASD that require an MRI scan.

37. My Itchy Skin Makes Me Sad

François, Sandy; Smith, Shelby; Sasaki, Jodie; Lawley, Leslie P.; and Chen, Suephy C.

Atopic dermatitis (AD) is a prevalent diagnosis in the pediatric population, which is exemplified by chronic pruritus. AD is a multifaceted diagnosis that can have an important impact on the quality of life (QoL) of children and their families, which can make management challenging at times.

A 5 year-old biracial boy with a history of AD since infancy presented to his 6 week follow up visit with his grandparents. The boy stated that his skin was very itchy and that he scratched it a lot. His itchiness made him sad and he said: "I can't take it anymore." Upon further questioning it was discovered that his itch had been severe and distracting enough that he was removed from school until the next school year. There was inconsistency in implementing daily treatments by parents. The father didn't believe the child had a skin disease and the birth of another child was expected in the coming weeks. Grandparents and parents all took part in managing the child's AD.

Eczematous, erythematous plaques with excoriation and fissures were present mainly on his foot, ankle, wrist, popliteal and antecubital fossa bilaterally. On the scalp, there were adherent scales and flakes. The review of other systems was within normal limits. Topical triamcinolone, bethametasone, and clobetasol as well as hydroxyzine were prescribed. Improvement is expected and there was an extensive discussion on skin care regimen with grandparents to attain proper symptomatic control. Systemic medication was also discussed if no improvement ensues.

This case demonstrates the importance of consideration of family dynamics and psychosocial impact on the child when managing AD. The emotional impact of AD on children can be significant. Assessing the child's adjustment within their development stage on how they manage their distress and partake in their care is imperative. Psychological strain can contribute to the disease progression. The coping challenges a family experiences need to be addressed with tailored interventions. The intervention can focus on parent-child dyad, parental dyad or other family members. Thorough education of all family members involved in the child's care is of utmost importance for optimal results.

38. Clinical Practice Guideline Adherence in Tympanostomy Tube Placement for Treatment of Recurrent Acute Otitis Media

Gaffney, Sierra; Boss, Emily, and Raol, Nikhila

INTRODUCTION: Tympanostomy tube placement is the most common surgical procedure in children and has been theorized as a source of healthcare overuse.

OBJECTIVES: To compare the frequency of acute otitis media (AOM) and healthcare utilization for children who underwent tympanostomy tube placement before or after being diagnosed with recurrent AOM (RAOM).

METHODS: We identified children born 2009-2013 in the MarketScan claims database who had at least one episode of AOM and underwent tympanostomy tube placement. We dichotomized children into two groups: 1) "post-RAOM": tympanostomy tube placement after 3 AOM episodes in 6 months or 4 AOM episodes in 12 months, with one in the last 6 months; and 2) "pre-RAOM": tympanostomy tube placement prior to meeting this criteria. We performed multivariable regression comparing healthcare utilization, as characterized by number of AOM episodes, antibiotic prescriptions, and visits to healthcare providers at one and two year follow up.

RESULTS: At one-year follow up, the pre-RAOM group had more AOM episodes (1.17 vs. 1.13, $p<0.0001$) and total antibiotics (1.43 v. 1.32, $p<0.0001$) than the post-RAOM group. The pre-RAOM group also had more visits to primary care providers (4.28 v. 3.52, $p<0.0001$), otolaryngologists (1.69 vs. 1.59, $p<0.0001$), and emergency departments (0.18 vs. 0.14, $p<0.0001$). These trends persisted at two-year follow up ($p<0.0001$).

CONCLUSIONS: Tympanostomy tube placement after RAOM diagnosis is associated with decreased healthcare utilization at one and two years of follow-up versus placement before RAOM diagnosis. Future research should elucidate patient, provider, and system-level factors that drive appropriate tympanostomy tube placement versus overuse.

39. Impact of Tympanostomy Tube Placement on Healthcare Utilization for Children with Recurrent Acute Otitis Media

Gaffney, Sierra; Boss, Emily; and Raol, Nikhila

OBJECTIVE: To compare healthcare utilization between children with recurrent acute otitis media (AOM) who underwent tympanostomy tube (TT) placement versus those who were managed medically.

METHODS: We identified children born 2009-2013 in the MarketScan claims database who met criteria for recurrent AOM and compared those treated with TT placement to those managed medically. We performed 3:1 matching on age, sex, geographic region, and date of recurrent AOM diagnosis to identify 145,002 pairs. We performed one-way analysis of variance to compare healthcare utilization, as characterized by number of AOM episodes, antibiotic prescriptions, and visits to health care providers at one and two years of follow up.

RESULTS: At one-year follow up, children treated with TTs had more infections (1.07 vs. 0.95, $p<0.0001$) and received more otic antibiotic prescriptions (0.59 vs. 0.06, $p<0.0001$) and total antibiotic prescriptions (1.40 vs. 0.89, $p<0.0001$), but fewer oral antibiotic prescriptions (0.81 vs. 0.83, $p=0.0002$). Individuals with TTs had more visits to primary care providers (3.47 vs. 3.09, $p<0.0001$), otolaryngologists (1.57 vs. 0.14, $p<0.0001$), and emergency departments (0.13 vs. 0.10, $p<0.0001$). At two-year follow up, the TT group had higher utilization across all outcomes ($p<0.0001$).

CONCLUSIONS: In the first two years after diagnosis of recurrent AOM, TT placement is associated with more episodes of AOM, visits to health care providers, and total antibiotic prescriptions. Given the cost and risk associated with surgical intervention, future research should seek to determine factors that drive these differences, as well as identify methods to decrease utilization following TT placement.

40. Evaluation of Left Ventricular Outflow Gradients During Staged Exercise Stress Echocardiography Helps Differentiate Patients with Hypertrophic Cardiomyopathy from Athletes and Normal Subjects

Gaitonde, Mansi; Jones, Shannon; McCracken, Courtney; Ferguson, Eric; Michelfelder, Erik; Sachdeva, Ritu; and Border, William

BACKGROUND: Elevated left ventricular outflow tract (LVOT) Doppler gradients during peak exercise can occur in patients with hypertrophic cardiomyopathy (HCM) as well as athletes and normal subjects. Our staged upright exercise protocol calls for detailed two-dimensional and Doppler imaging at rest and during each stage of exercise until peak exercise, allowing us to evaluate the mechanism of LVOT obstruction at each stage. We sought to determine whether this staged approach helps differentiate HCM patients from athletes and normal subjects who develop elevated gradients with exercise.

METHODS: We reviewed records of patients <22 years who underwent a stress echocardiogram from Jan 2009 - Oct 2017 at our center. We identified all patients with gene-positive HCM, those diagnosed with athlete's heart, and normal subjects. We then selected subjects with no significant LVOT gradient at rest while supine who subsequently developed a LVOT peak gradient of at least 25 mmHg at peak exercise. We measured LVOT peak gradient, velocity time integral (VTI), acceleration time (AT), and deceleration time (DT) at rest while supine, at submaximal stages, and at peak exercise. T-test, ANOVA, and Tukey-Kramer method were used for statistical analysis with p-value set at < 0.05.

RESULTS: Compared to athletes (n=10) and normal subjects (n=10), respectively, HCM patients (n=10) had a difference in LVOT peak gradients at rest (p=0.019; p=0.007), stage 1 of exercise (p=0.002; p<0.001), and peak exercise (p=0.051; 0.003), as well as a difference in the change in LVOT peak gradient from rest to stage 1 (p=0.016; p=0.015) and from rest to peak (p=0.038; p<0.001). The VTI of the LVOT doppler in HCM patients was different at rest, stage 1 of exercise, and peak exercise compared to athletes and normal patients.

CONCLUSION: HCM patients who develop elevated LVOT gradients at peak exercise typically manifest early obstruction in the submaximal stages of exercise, which helps to differentiate them from athletes and normal subjects. This supports the use of staged exercise echocardiography in these patients.

41. Towards an HIV Cure: Screening For Agents That Selectively Disrupt Establishment of the HIV Reservoir in Primary Myeloid Cells

Gavegnano, Christina; Shepard, Caitlin; and Kim, Baek.

BACKGROUND: HIV-1 infects both activated CD4+ T cells and non-dividing myeloid cells (macrophages) during pathogenesis. HIV-1 infection induces death of activated CD4+ T cells, leading to immune deficiency of infected hosts, but HIV-1 infected macrophages survive for a long period of time, becoming key long-living HIV-1 reservoirs contributing to HIV-1 persistence. While HIV-1 infected activated CD4+ T cells display robust viral production, including cell-cycle arrest and cell death, HIV-1 replication kinetics in macrophages is very slow; these delayed kinetics likely allows HIV-infected macrophages to survive, thereby facilitating a long-lived macrophage-derived reservoir. We screened a library of ~2,500 agents including FDA approved, late phase clinical agents, or natural products for their ability to stimulate the suppressed viral replication and production, which can interfere with the long-term survival of HIV-1 infected myeloid reservoirs. Agents with this unique profile can be developed as anti-HIV-1 therapies that can specifically target the long-living myeloid reservoirs for HIV-1 cure.

METHODS: Four donor pooled HIV-negative monocyte-derived-macrophage donors were pre-treated with 10 μ M library compounds for 2hr, and then transduced with DHIV-3 (HIV-1 single-cycle vector system with a GFP reporter). Transduced cells were fixed (4% paraformaldehyde) and stained with DAPI (nuclei) at 48hr post transduction and analyzed by Cytation3 to quantify GFP expressing cells normalized to cell count. Controls were performed in triplicate (negative control; no drug, vector only; positive control viral like particles (VLP)+vector, which increase dNTP substrates for the HIV-1 reverse transcriptase, thereby resulting in GFP expression at 48hr post transduction).

RESULTS: Of >1,000 compounds screened to date, agents demonstrating GFP increase above positive control (VLP) were: 46 demonstrated 2-5 fold increase; 21 demonstrated 5-10 fold increase; 14 demonstrated 10-20 fold increase; 9 demonstrated >20 fold increase without apparent toxicity (no significant reduction in total cell counts).

CONCLUSIONS: For the first time, we have identified safe, potent agents that modulate key events involved in establishment and maintenance of the HIV reservoir in macrophages. These agents will be further evaluated for their ability to modulate key events involved in HIV persistence and barriers to a cure in clinically relevant cells and eventually in humans.

42. Atlanta Sickle Cell Summer Research Program

Gee, Beatrice; and Joiner, Clinton

BACKGROUND: There is a substantial shortage of physicians and biomedical researchers from under-represented groups. Participation of under-represented groups in medicine and biomedical research may be enhanced by early exposure of minority youth to quality research experiences and

ongoing mentoring and training experiences. The Excellence in Hemoglobinopathies Research Award (EHRA) program (NIH/NHLBI 2013 - 18) supports the development of studies that will accelerate high-impact multi-disciplinary basic and translational research in the hemoglobinopathies and facilitate maximal collaborations among basic and translational scientists and clinical hematologists. As part of the Emory/Pittsburgh/Vanderbilt Center of Excellence in Hemoglobinopathies Research, we conducted the Atlanta Sickle Cell Summer Research Program.

METHODS: We provided high school students from under-represented groups an eight-week mentored research experience with active sickle cell researchers at Emory, Morehouse School of Medicine and Georgia Institute of Technology. Students were recruited from Metropolitan Atlanta high schools through e-mail and brochure distribution and in-person meetings. Online applications were accepted from February 1 – April 1 of each year. Highest ranking applicants were invited to an interview with faculty and program staff. In addition, students attended didactic sessions about blood and sickle cell pathophysiology, had weekly readings and writing assignments, were introduced to hypothesis generation and study design, learned basic biostatistical methods prepared and presented an oral presentation.

RESULTS: We hosted 3-4 students each year. Twelve students were trained from 2013 -17 (three returned for a second year). All of the students who graduated and begun college, and most are in STEM fields. One of our trainees received the Gates Millennium Scholarship. We found that outreach to local public high schools was challenging. Eight-week long programs conflict with summer plans for some students. Plans for inter-campus transportation need to be included.

CONCLUSIONS: To help develop early interest in hematology careers in minority youth, we conducted a summer research program for high school students focused on hands-on sickle cell research as part of the NIH/NHLBI EHRA program. This funding mechanism will not be renewed. Our future direction is to apply for alternative training program funding, ex. NIH R25.

43. A Metabolic Approach Promotes the Maturation of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Gentillon, Cinsley; Duan, Meixue; Yu, Wen-Mei; Jha, Rajneesh; Gibson, Greg; Qu, Cheng-Kui; Brown, Lou Ann; and Xu, Chunhui

BACKGROUND: Immature phenotypes including immature metabolic status of cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) limit their utility in clinical application and basic research. It is widely known that a transition in energy metabolism is implicated in CM differentiation and development. While fetal cardiomyocytes rely on glycolysis to produce most energy, adult cardiomyocytes use predominantly fatty acid oxidation (FAO). Using this metabolic hallmark of CM maturation, we investigated a platform that combines 3D cell cultivation and metabolic regulation.

METHODS & RESULTS: Cardiac spheres of highly-enriched hiPSC-CMs were generated from cardiac-differentiated cultures and treated with a combination of molecules that target key pathways

involved in the energy metabolism of cardiomyocytes. Compared with DMSO-treated hiPSC-CMs, the maturation factor-treated cells had significantly increased FAO and mitochondrial respiratory capacity as detected using a Seahorse XF-24 extracellular flux analyzer. The treated cultures had increased mitochondrial content and contained more cells with mitochondrial distribution throughout the cells, features of more mature CMs. Consistent with these observations, a number of transcriptional regulators of mitochondrial metabolic processes were upregulated in the treated hiPSC-CMs as detected by qRT-PCR and RNA sequencing. Furthermore, these cells had significantly increased Ca²⁺ transient kinetics, a functional feature for more mature CMs.

CONCLUSIONS: Combining 3D tissue engineering with metabolic regulation promotes FAO, increases mitochondrial function and content, and improves Ca²⁺ handling properties in hiPSC-CMs.

44. Difference between SF₆ and N₂ Multiple Breath Washout Kinetics is Due to N₂ Back Diffusion and Error in N₂ Offset

Guglani, Lokesh; Kasi, Ajay; Starks, Miah; Pedersen, Knud; Nielsen, Jørgen G; and Weiner, Daniel J.

BACKGROUND: Measurement of Lung Clearance Index (LCI) by Multiple Breath Washout (MBW) is a sensitive method for monitoring of lung disease in Cystic Fibrosis (CF). To identify differences between N₂-MBW and SF₆-MBW, we connected two gas analysis systems in series to provide truly simultaneous measurements and with no other differences other than the gas used.

METHODS: Non-smoking healthy controls (HC) and subjects with CF were recruited at two institutions. The Exhalyzer-D (for N₂ measurement) was connected in series with the Innocor (for SF₆ measurement). Subjects washed in SF₆ from a Douglas bag with tidal breathing, and washed out SF₆ and nitrogen with 100% oxygen provided as bias flow. Washout of both gases was continued past the LCI point (1/40th of equilibration concentration) in triplicate.

RESULTS: N₂-MBW (compared to SF₆-MBW) resulted in higher Cumulative Exhaled Volume (CEV), FRC, and LCI in HC subjects (p<0.001 for all comparisons). All N₂-MBW parameters were also significantly higher than SF₆-MBW parameters in CF subjects (p<0.001 for all comparisons). After recalculation with a common FRC, N₂MBW-LCI was higher than SF₆MBW-LCI in CF subjects (19.73 vs. 11.39; p<0.001) and in HC (8.12 vs. 6.78; p<0.001). Adjusting for N₂ back diffusion and an offset error in the nitrogen measurement resulted in near complete agreement between the two methodologies.

CONCLUSION: We found significant differences in LCI and FRC measurements using two different gases for MBW. This may have significant implications for the future use and interpretation of LCI data in clinical trials and routine clinical care.

45. Impact of Early Life Injury on Microglial Activation in Periaqueductal Grey and Sickness Behavior After Later Life Immune Challenge

Hanus, Lauren; and Murphy, Anne

Premature babies, who make up 16.5% of the babies born, experience about 14 painful procedure each day in the Neonatal Intensive Care Unit (NICU); 65% of the procedures are performed without analgesics. We have previously shown that a single inflammatory insult on the day of birth significantly alters opioidergic neural circuits within the midbrain PAG, permanently changing subsequent responses to pain and stress. Microglia, the resident immune cells of the brain, are particularly vulnerable to early life perturbations, which may have profound consequences for future immune responses. However to date, the impact of early life pain on microglia expression and activation are unknown. On the day of birth, male and female rat pups received an injection of the inflammatory agent carrageenan (CGN) into the hindpaw or were handled. In adulthood (postnatal day 60-90), rats were implanted with a Thermicron iButton to monitor autonomic output, and 10 days later, received an injection of the endotoxin lipopolysaccharide (LPS) to induce an immune challenge. Sickness behavior was assessed six hours after LPS injection with a modified rat grimace scale. Tissue was then collected and stained for microglia using immunohistochemistry. Microglia were characterized as amoeboid, activated or quiescent based on morphology. No differences microglial colonization in the PAG were observed for early life pain versus control rats; similarly, no sex differences were observed. After an immune challenge, the rats that received early life CGN had more glial activation in the PAG than the rats who were handled, indicating a lower threshold for activation. Rats with early life CGN also had more sickness behavior regardless of sex. Female CGN treated rats had lower febrile response than their handled counterparts, and more closely matched the males, which did not differ from each other. While early life injury does not affect the density of microglial cells in the PAG, the change in response to an immune challenge is seen not only in the microglial activation in the brain, but also in behavior and physiological responses.

46. Diagnostic Value of Echocardiography Speckle Tracking and Magnetic Resonance Feature Tracking in Children with Myocarditis

Hashemi, Sassan; Sallee III, Denver; Parks, W. James; and Slesnick, Timothy

INTRODUCTION: Myocarditis is an important cause of morbidity and mortality in children. Myocardial strain has been shown to be a sensitive measure for myocardial function, including sub-clinical changes. We sought to compare the diagnostic value of echocardiography (echo) speckle tracking (ST) and cardiac magnetic resonance (CMR) feature tracking (FT) indices in children diagnosed with myocarditis.

METHODS: All patients diagnosed with myocarditis between 2014 and 2016 who had an echo and a CMR ≤ 5 days apart were included. Demographic and imaging characteristics were reviewed. Maximum troponin and BNP levels during the course of hospitalization were extracted. Late Gadolinium enhancement (LGE) burden was quantified (mild $< 25\%$, 25 \leq moderate $< 50\%$, severe $\geq 50\%$) from CMR. FT and ST analyses were performed in short axis and 4-chamber views to

calculate global circumferential (GCS), radial (GRS) and longitudinal (GLS) strain of the left ventricle.

RESULTS: Eighteen patients (Male=14) with a median age of 15.5 years (range: 8.4-17.7) met inclusion criteria. There was a median of 1 day (0-4) between echo and CMR. LGE was positive in 13 patients with median burden of 23% (0-75%). Functional characteristics are summarized in table 1. Left ventricular ejection fraction and GLS were not statistically different between echo and CMR ($P=0.91$ and 0.44), whereas GCS and GRS showed a significant difference (both $P=0.02$). Ejection fraction showed stronger correlation with GCS for both modalities. Unlike GLS, echo GCS ($P=0.01$) and GRS ($P=0.03$) and CMR GCS ($P=0.03$) and GRS ($P=0.02$) showed significant differences between patients with preserved and reduced ejection fraction. Correlation between CMR-GLS and LGE level was the strongest between functional indices and LGE, maximum troponin or BNP levels.

CONCLUSION: Retrospective myocardial strain analysis using both ST and FT in children with myocarditis is feasible. GCS had the best correlation with ejection fraction for both modalities and GLS was the only index to not show significant differences between preserved and reduced ejection fraction groups. CMR-GLS showed the strongest correlation with LGE burden compared to other functional indices and biomarkers. These findings may suggest the sequence in which strain indices change in this patient population.

47. The Pediatric Unexplained Encephalitis Study (PUES): A Prospective Cohort Analysis Evaluating Next-generation Sequencing as a Diagnostic Modality

Haston, Julia; Rostad, Christina; Wiley, Michael; Pratt, Catherine; Prieto, Karla; Shane, Andi; Palacios, Gustavo; and McElroy, Anita

BACKGROUND: Encephalitis can be associated with focal and global neurologic dysfunction resulting in significant morbidity and mortality in children. Although viruses are often implicated, an etiology is not identified in the majority of cases. Next-generation sequencing (NGS) is a high-throughput metagenomics-based sequencing technique that may enhance the detection of novel or low frequency pathogens.

METHODS: Hospitalized immunocompetent patients aged 6 months-18 years with encephalitis of unidentified etiology were eligible for enrollment. Demographic, historical, and clinical information was obtained using a standardized questionnaire. Serum or plasma and cerebrospinal fluid (CSF) samples were subjected to NGS. Pathogens were identified by comparing the nucleic acid sequences from the samples with online databases of sequences.

RESULTS: Twenty patients aged 6 months-17 years (median 7.5 years) and 50% female were enrolled from 2013 to 2017. NGS identified non-human nucleic acid sequences of significant frequency in 4 patients. Those identified by NGS were *Cladophialophora bantiana* in CSF, *Mycoplasma bovis* in both serum and CSF, and Parvovirus B19 in CSF, as well as tobacco mosaic virus and human bocavirus, which were presumed contaminants. Two organisms diagnosed by

conventional CSF PCR, *Neisseria meningitidis* and *Balamuthia mandrillaris*, were initially not identified by NGS, but were detected in the NGS data in retrospective analysis. One additional patient was found to have positive IgM serology for California Encephalitis Virus, but molecular diagnosis was not achieved.

CONCLUSIONS: We describe a prospective cohort analysis evaluating NGS as a diagnostic tool for children with unexplained encephalitis. Limitations of NGS in this study included detection of contaminants and difficulty distinguishing low levels of pathogen nucleic acid sequences from background. However, multiple putative pathogens were identified suggesting that NGS could facilitate pathogen discovery and diagnosis.

48. Post-transplant Survival Analysis for Pediatric Kidney Transplant Recipients

He, Xi; Xie, Yao; Sokol, Joel; and Keskinocak, Pinar

We aim to build a statistical model that accurately predicts the post-transplant survival curves for pediatric organ transplant recipients and identify the most important variables that determine the survival curves. Pediatric transplant recipients are less commonly studied in the existing literature, while models developed for the general transplant recipients may not apply due to the very different physiological conditions of the patients.

We consider a dataset consisting of 19,291 pediatric patients who received kidney transplants in the U.S. from 1987 to 2012. The dataset includes living and deceased donors as well as censored recipient data, which means these recipients were lost track during of the course of the study, and 94.19% of recipient data are censored. The data contains 485 features on either the patient or the donor, with various percentages of missing values. The data are provided by UNOS (United Network for Organ Sharing), a non-profit, scientific and educational organization that administers the OPTN (Organ Procurement and Transplant Network) in the United States.

Since pediatric transplant recipients include newborns as well as teenagers, it is important to study different age groups separately. In addition, the survival curves for pediatric recipients receiving organs from adult and pediatric donors are quite different. Therefore, we examine how to group the pediatric recipients by recipient age and donor age, so that their survival curves are similar within groups, and different across groups. Using statistical methods including sequential two-sample tests, as well as machine learning clustering techniques such as k-means clustering and decision tree, we identify multiple groups (as a combination of recipient age and donor age). For each of these groups, we performed survival analysis using Cox proportional hazard model and random survival forests (RSF). We measure the performance using the Brier score and Harrell's Concordance index.

Furthermore, we use statistical variable selection to determine variables that are most important for post-transplant survival curves for pediatric patients, and found recipient age and weight among the most important variables. In this study, we focus on kidney transplant data but the methodologies are generic and are applicable to other types of organs.

49. MiR-486 is an Epigenetic Regulator of the Pathological Progression of Duchenne Muscular Dystrophy

Hightower, Rylie; and Alexander, Matthew

Duchenne muscular dystrophy (DMD) affects 1 in 5000 live male births making it the most common form of muscular dystrophy worldwide. Patients with this X-linked progressive neuromuscular disorder develop muscle loss, ambulation loss, cardiac arrhythmias, and respiratory complications. DMD is caused by non-functional mutations in the DYSTROPHIN (DMD) gene, ultimately resulting in myofiber membrane breakdown, myofiber death, and whole muscle atrophy. Although all patients experience many similar cardinal disease manifestations, there remains a wide spectrum of phenotypic variability between patients regarding onset and severity of symptoms, implying contribution of biological factors other than DMD gene mutations to pathological disease progression. MicroRNAs have shown to play an important role in muscle development and maintenance. It has been previously demonstrated that miR-486 expression is significantly decreased in both DMD patients and mouse models. Data from our lab suggests that overexpression of miR-486 in dystrophin-deficient mice can ameliorate disease pathology. From this, we hypothesized that miR-486 is a significant epigenetic regulator of disease pathology in DMD. Locomotive, histological, and metabolic analyses were used to assess muscle-specific transgenic overexpression of miR-486 in dystrophin-deficient mice as well as global knockout of miR-486 in both WT and dystrophin-deficient mice. We observed that miR-486 overexpression improves serum creatine kinase levels, improved muscle architecture, reduced centralized myonuclei, and increased physical activity in dystrophin-deficient adult male mice. MiR-486 global knockout resulted in significant disruption of muscle architecture, decreased physical activity, and cardiac and metabolic defects compared to WT controls. Based on these results, additional experiments focused on the mechanistic characterization of miR-486 and its mRNA targets will help determine the applicability and use of miR-486 as a biomarker and therapeutic agent for DMD.

50. Administration of KPT-350 Ameliorates Duchenne muscular dystrophy Symptoms in Dystrophic Zebrafish and Mice

Hightower, Rylie; Gibbs, Devin; Lee, Christopher; Spinazzola, Janelle; Widrick, Jeffrey; Tamir, Sharon; Cochran, Shelton; Chang, Hua; Landesman, Yosef; Kunkel, Louis; and Alexander, Matthew

OBJECTIVE: This study evaluated the effectiveness of the Selective Inhibitor of Nuclear Export (SINE) compound KPT-350 in zebrafish and mouse models of Duchenne muscular dystrophy (DMD).

BACKGROUND: DMD is an X-linked disorder that afflicts approximately 1:5000 live male births, making it the most common form of muscular dystrophy worldwide. The nuclear export protein XPO1/CRM1 is a promising target for the treatment of neurological disorders with inflammatory pathology such as DMD. KPT-350 targets XPO1, a nuclear exportin protein that regulates the localization and function of >200 cargo proteins, including transcription factors that regulate inflammation and neurotoxicity. KPT-350 is a potent, small molecule, orally available, slowly

reversible inhibitor of XPO1, and KPT-350 administration has been shown to improve phenotypes in other rodent models of neuromuscular disease.

DESIGN/METHODS: To assess the short-term effect of KPT-350 treatment on dystrophic disease phenotype and muscle architecture, sapje zebrafish (severe model of DMD) embryos were treated from 1 to 5 days post-fertilization (dpf) with vehicle, 1.25 μ M KPT-350, 2.5 μ M KPT-350, or 2.5 μ M aminophylline (positive control). Additionally, we tested oral KPT-350 (5 mg/kg body weight) in adult mdx (DBA2J) and WT control mice 3 times a week for 8 weeks in a double-blinded fashion.

RESULTS: In short-term and long-term treatment studies, KPT-350-treated sapje zebrafish showed significant prevention of the muscle degeneration pathology associated with dystrophin-deficiency and improved overall muscle architecture as determined by histological analysis of myosin heavy chains. In mdx (DBA2J) mice, KPT-350 treatment blocked muscle inflammation, improved movement distance and velocity before forced treadmill running, improved muscle histology, normalized cytokine biomarkers, and reduced overall dystrophic symptoms compared to vehicle controls.

CONCLUSIONS: Our studies demonstrate that KPT-350 is a promising small molecule therapeutic that can attenuate DMD symptoms.

51. Primary Versus Secondary Anastomosis in Intestinal Atresia

Hillyer, Margot; Baxter, Katherine; Clifton, Matthew; Gillespie, Scott; Bryan, Leah; Travers, Curtis; and Raval, Mehul

PURPOSE: Neonates with intestinal atresia undergo operative repair using one of two techniques: primary anastomosis (PA) or ostomy creation with secondary anastomosis (SA). This study attempts to compare the factors influencing surgical decision-making and the outcomes of these techniques.

METHODS: We conducted a retrospective cohort study of 104 neonates between 2009-2015 at an 18-surgeon, 2-hospital system. Associations between surgical approach and patient characteristics, operative details, and outcomes were analyzed using univariate comparison and bivariable/multivariable logistic regression models. Surgeon-level preferences, defined as performing >50% PA or SA, were further assessed using logistic regression.

RESULTS: Of 104 patients, 73 (70.2%) underwent PA. These neonates had a shorter length of stay (26.5 vs. 82 days, $p < 0.001$), shorter duration of total parenteral nutrition (19 vs. 62 days, $p < 0.001$), and fewer readmissions within 1-year (32.1% vs. 67.9%, $p = 0.002$). Factors associated with undergoing PA on multivariable regression analysis included 1-minute Apgar (6-10, Odds Ratio (OR) 3.3, 95% Confidence Interval (CI) 1.08-10), non-emergent presentation (OR 7.73, 95% CI 1.18 - 50.6), and atresia location (jejunum OR 21.27, 95% CI 3.77-119.9). At the surgeon-level, utilization of PA varied from 35.7% to 100%. Emergent presentation was the only variable associated with surgeon preference ($p = 0.02$). No other demographic or operative details were associated with preference ($p > 0.05$) suggesting that surgeon preference contributes to procedural selection.

CONCLUSION: PA is associated with better outcomes than SA. While there are multiple clinical factors that influence approach selection, surgeon preference appears to be an independent factor illuminating an opportunity to establish best practices and decrease variation in care.

52. Vaginal Delivery Activates Hypothalamic Vasopressin Neurons in the Perinatal Mouse Brain

Hoffiz, Yarely; Castillo-Ruiz, Alexandra; and Forger, Nancy G.

Birth is a dramatic event; the abrupt separation from the placenta and the rapid transition from the uterine environment forces the fetus to make quick physiological adjustments in peripheral organs in order to survive ex-utero. It is likely that the brain also experiences dynamic changes at birth, but how the brain responds to these challenges around the time of birth has not been explored. Here, we directly addressed this question by studying the pattern of neural activation in the perinatal mouse forebrain. We established timed-pregnancies in C57BL/6 mice and collected the brains of male and female offspring in-utero at embryonic day 18.5, and ex-utero at 1h, 3h and 1 day after birth following vaginal delivery. Brains were processed for the immunohistochemical detection of the immediate early gene product, c-Fos, as a marker of neural activation. We found that a vaginal birth triggers activation in discrete hypothalamic areas: the suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus showed significant changes in c-Fos labeling, with neural activation increasing up to 500% 3h after birth compared to one day before birth, 1h or 1 day after birth. These data suggest that specific hypothalamic areas are particularly sensitive to stimuli at birth. We next analyzed the activation pattern of subsets of cells in these regions that are known to play important roles at birth. Immunofluorescent double labeling of vasopressin or oxytocin with c-Fos, showed that a vaginal birth triggers the activation of vasopressin neurons, but not oxytocin neurons in the hypothalamus at 3h postpartum. Vasopressin is a neuropeptide involved in water balance peripherally, and in the stress response and social behaviors when released centrally, and has been shown to be massively released after a vaginal but not a cesarean delivery. Given the high rate of cesarean deliveries in the United States and other countries, we are also in the process of analyzing how alterations in birth mode affect the perinatal pattern of neural activation.

53. Developing a Smart Continuous Monitoring System for Feeding Intolerance in the Pediatric ICU

Hoffman, Ryan; Novack, Jack; Chanani, Nikhil; and Wang, May

Good nutrition is central to the management of hospitalized children, and even more critical for the sick infant. It is well recognized that enteral (GI tract) feeds are to be used whenever possible, as this mode of feeding is associated with the best growth and development, and fewer complications such as liver disease and infection that can occur with parenteral nutrition. However, initiating and advancing enteral nutrition in the hospitalized child is not without its own issues, as perfusion to the GI tract may be compromised, putting these patients at additional risk of necrotic disease. The

premature infant and children with cardiac disease are at particular risk. Currently, there is no good method to determine feeding tolerance in this patient population. Practitioners observe for abdominal distension, pain, bloody stools, infection, etc. These findings are late in the course of illness, and may not even be representative of the disease in question. A method to continuously evaluate for enteral feeding tolerance (EFI) would be of great benefit to the care and management of these patients. The goal of this project is to develop a clinical decision support system with advanced machine learning algorithms that will enable physicians to predict which patients will develop or are developing EFI.

54. Development of EIA Assays Using Different Antigens to Measure Antibody Response to RSV Infection in Children

Jadhao, Samadhan; Ha, Binh; McCracken, Courtney; Kumar, Vishnu; Hartert, Tina; and Anderson, Larry

BACKGROUND: Respiratory syncytial virus (RSV) is a common infection in infancy, with nearly all children experiencing infection by two years of age. Hospitalization due to RSV lower respiratory tract disease range from 0.5% to 2.0%. Among infected children, about 50% to 90% develop bronchiolitis and 5% to 40% develop pneumonia. Assays to detect and quantify antibodies to RSV are important to understand the immune response to RSV infection and vaccines but also to determine primed versus naïve status for accurate evaluation of the effect of RSV vaccination, independent of an effect of prior infection, on RSV disease.

METHODS: We developed enzyme immunoassays (EIAs) with pooled RSV A and B lysate (there are two major antigenic groups of RSV strains, A and B), recombinant expressed F protein, recombinant expressed G proteins, one for group A and one for group B. The G protein is antigenically distinct while the F protein is highly conserved between the two groups. We compare the ability of these four assays to detect RSV antibodies and the titer of detected antibody in plasma specimens from 120 young children with prior documented RSV infection and over 120 young children with no documented past RSV infection. With these EIAs, we compare OD490 values from patient's plasma against the RSV antigen and control antigen to determine a positive response and estimate titer from a standard curve run the same day using SAS based automated regression analysis program developed by the Emory University Pediatric Biostatistics Core.

RESULTS: We will present results on evaluation of these four distinct RSV antibody EIAs. The pooled lysate and anti-F protein EIAs detect most documented infections while the G protein EIAs are less sensitive and provide information RSV specific infection. Over 90% of the plasma, specimens from children with prior documented RSV infection were positive for RSV antibody by EIA based on pooled RSV A and B lysate or recombinant expressed F protein antigen.

INTERPRETATION: The RSV antibody ELISA developed would help to identify RSV primed and naïve state and infecting strain of RSV in children and help estimate antibody response to RSV vaccines.

55. Reprogramming of Human Induced Pluripotent Stem Cells Using Thymus-derived Fibroblasts for the Recapitulation of Familial Long QT Syndrome In-a-dish

Jha, Rajneesh; Deshpande, Shriprasad; Maher, Kevin; and Xu, Chunhui

Cardiomyocytes derived from induced pluripotent stem cells (iPSCs) are being increasingly investigated to improve our understanding of disease modeling, toxicity testing and regenerative therapies for congenital heart disease (CHD). During placement of ventricular assist device, the thymus is resected and discarded per standard procedure. Therefore, we tested if thymus can be a cell source for iPSC reprogramming. A newborn with KCNQ1 and other mutations developed familial long QT syndrome and severe cardiomyopathy at 4 months of age. The patient showed decompensated heart failure with medical therapies and warranted an escalation of support by removing a portion of thymus and placement of ventricular assist device. A small section of the thymic tissue (<3 mm diameter) was cultured in human dermal fibroblast medium supplemented with Amphotericin B. After 4-5 days, fibroblast outgrowths were observed, and expanded until they became homogenous fibroblastic cultures. Early passage fibroblast suspension mixed with 4 episomal plasmids (pCXLE-hOCT3/4-shp53-F, pCXLE-hSOX2-KLF4, pCXLE-hLMYC-LYN28, and pCXLE-EGFP) was nucleofected using program P022. Cells were then transferred to 0.5% gelatin-coated plates and fed daily with stem cell culture medium supplemented with bFGF and human iPS Cell Boost Supplement II until iPSC colonies started to appear. At day 15 post-nucleofection, stem cell-like colonies started appearing which were further expanded until day 21. Clones were confirmed by immunofluorescence staining with Tra1-60 antibody and were picked for further expansion either on feeders or in a feeder-free mTeSR medium. iPSCs were further characterized by expression of pluripotent markers and differentiated to cardiomyocytes using growth-factor guided protocol. These cardiomyocytes are being used for electrophysiological testing to examine the long QT phenotype. This is the first report of the successful derivation of iPSCs from thymus fibroblasts and could provide a convenient cell source for reprogramming especially in a patient whose tissue is often discarded during CHD surgeries.

56. Delivery of Novel Particulate Measles Vaccine via Oral Dissolving Films (ODF)

Joshi, Devyani; Bajaj, Lotika; Gala, Rikhav; Popescu, Carmen; Knipp, Gregory; McCain, Robyn; Ubale, Ruhi; Addo, Richard; Bhowmik, Tuhin; and D'Souza, Martin

INTRODUCTION: We formulated oral measles vaccines for needle-less delivery for better patient-compliance. Goal was to explore potential of oral disintegrating films (ODF) loaded with measles vaccine microparticles for long-lasting immune response.

METHODS: The measles virus antigen was formulated using pre-cross-linked bovine serum albumin (BSA). The solution was spray dried to form microparticles containing the vaccine antigen using Buchi Spray Dryer B-290. The ODF Film formulation was cast into thin strips using the BYK-Gardner equipment and air dried. The ODF vaccine was tested in-vivo in pigs by buccal delivery. Blood serum samples were collected every 2 weeks and antigen specific ELISA was performed to quantify amount of specific antibody.

RESULTS: The size range of microparticles was 400-1200 nm. The desired film strength & disintegration properties were obtained by pre-gelatinized hydroxypropyl pea starch & Lycoat RS720. Significant increase in specific antibodies against measles was observed after 4 weeks of dosing and remained elevated until end of 6 week. There was significant increase in serum IgG levels when compared with serum IgG levels pre-dosing and post-dosing.

CONCLUSION: Buccal delivery of ODF loaded with vaccine microparticles is promising immunization delivery system.

TRANSLATIONAL RELEVANCE: ODFs are inexpensive and effective means to deliver vaccines orally without use of needles and will help to increase patient compliance.

57. Effects of Literacy and IQ on Adherence, Quality of Life and Perceptions of Medication Barriers in Pediatric Renal Transplant Recipients

Kamel, Margret; Wilkerson, Alexandria; McCracken, Courtney; Alexander, Neneka; Gazmararian, Julie; and George, Roshan

INTRODUCTION: An adequate level of literacy is essential to comprehend information given by healthcare providers and to practice sustained behaviors needed for successful adherence and favorable outcomes. This project examines associations between literacy, IQ, adherence and perception of barriers to taking medication in pediatric kidney transplant recipients.

METHODS: Data were collected from subjects (18 parents and 41 patients), who were ≥ 6 months post-transplant with a stable, functioning allograft. Literacy in post renal transplant adolescents and their parents was assessed using Short test of functional health literacy (S-TOFHLA) and Newest Vital Signs (NVS). Barriers to adherence were assessed utilizing Adolescent and Parent Medication Barriers Scales (AMBS and PMBS). Intellectual functioning was assessed during pre-transplant evaluations. Clinical data including rejection history and hospitalization was also analyzed.

RESULTS: Patients' ages ranged from 15-21 years, with 56% males; 51% Caucasian, 34% African Americans. Among patients, 72% had inadequate numeracy literacy while 97% had adequate health literacy. Among parents, 65% had inadequate numeracy literacy but 96% had adequate health literacy. Patients with inadequate health numeracy were transplanted younger, and been transplanted for a significantly longer period. Patients with inadequate health numeracy were more likely to have below average IQ scores. Drug adherent patients reported more adherence barriers ($p=0.003$), greater disease frustration/ingestion issues, ($p=0.008$) and poorer regimen adaptation ($p=0.011$). Patients showing greater adherence reported greater ingestion issues ($p=0.008$), regimen adaptation/cognitive issues ($p=0.011$) and recognition of barriers ($p=0.03$). Among parents, numeracy literacy negatively correlated with perception of ingestion issues for their child ($r=-0.74$). Patients with adequate numeracy had more hospitalizations but lower rejection rates (0 vs 21.4%).

CONCLUSION: A majority of patients/parents showed inadequate numeracy literacy, despite adequate health literacy. Adherent patients are likely overcoming more barriers related to disease

frustration and medication ingestion; and adolescents with lower IQs are at particular risk. Streamlining medication education and actively assessing medication ingestion barriers, even in adherent patients, is vital. Numeracy literacy in patients with complex medical conditions (especially those with lower IQ) is a hidden barrier, which needs to be precisely targeted and overcome to improve long-term outcomes.

58. Single Cell Analyses of Human Antibody Responses to *Vibrio cholerae*

Kauffman, Robert; Adekunle, Oluwaseyi; Jun, Kong; Bhuiyan, Taufiqur; Ryan, Edward; Qadri, Firdausi; Harris, Jason; and Wrammert, Jens

Cholera is a severe diarrheal disease caused by *Vibrio cholerae*. Cholera affects more than three million people annually resulting in an estimated 100,000 deaths. It is also responsible for deadly epidemics as recently observed in Yemen. Children under five years of age represent the greatest burden of disease and are particularly susceptible to cholera, which can result in death within hours if untreated. This is due in part to the fact that children have limited naturally acquired immunity and currently available oral cholera vaccines have reduced efficacy in young children. Intriguingly, in contrast to vaccination, adult cholera infection induces long lasting immunity, likely mediated by intestinal antibodies. To further understand humoral immunity against cholera, we used single-cell expression cloning to generate panels of human monoclonal antibodies (mAbs) from acutely induced plasmablasts isolated from patients in Bangladesh. mAbs largely targeted the dominant antigens cholera toxin and lipopolysaccharide (LPS). Notably, while LPS responses targeted the O-specific polysaccharide moiety, mAbs varied in serotype specificity and functional characteristics. Unexpectedly, despite all patients being infected by the Ogawa serotype, isolated mAbs from one patient preferentially bound to the Inaba serotype, which had been almost undetectable in circulation for five years. Inaba selective mAbs were characterized by high levels of somatic hypermutation. These findings suggest that cholera can generate immunologic memory and induce significant somatic hypermutation in response to this polysaccharide antigen. To expand on these findings we generated a panel of isotype and subclass variants from six LPS specific mAbs, representing a range of affinity and serotype specificity. mAbs were expressed as IgG1, IgG2, IgG3, IgG4, pentameric IgM, as well as the monomeric and dimeric forms of IgA1 and IgA2, which dominate in the intestine. This analysis provided detailed insight into how immunoglobulin isotype and subclass impacts functional characteristics such as agglutination and vibriocidal activity. Moreover, using live-cell microscopy and soft-agar migration assays, we provide novel insight into the impact of these antibody variants on bacterial propulsion. Collectively these data inform future vaccine design that aims to generate a highly durable and potent antibody response similar to that of natural infection.

59. Pediatric Nanomedicines for Synergistic Combination Therapy of Childhood Leukemias

Kelvin, James; Perdue, Lacey; and Dreaden, Erik

Combination chemotherapies have greatly improved treatment outcomes in leukemia patients; however, conventional approaches to their delivery often ignore ratio-dependent drug interactions that can either synergize with - or antagonize - cell killing when local concentrations of drug fluctuate following administration. One approach to overcome such variability is to administer a fixed ratio of drugs via an engineered nanoscale drug carrier such as liposomal Vyxeos, which improves overall survival in a subset of adult leukemia patients via prolonged maintenance of a tumor-toxic, pairwise drug ratio. Recently, we developed a high-throughput combinatorial screening approach that extends this strategy through the identification of triplet drug combinations in which tyrosine kinase inhibition synergizes with - or antagonizes - leukemia cell killing in response to frontline cytotoxic chemotherapy. We identified highly conserved regions of both synergy and antagonism among more than 300 unique drug combinations tested in a panel of B-ALL cells and here, we describe the discovery of these tumor-toxic drug combinations and recent efforts to facilitate their conditional delivery to leukemia cells via nanoscale drug carriers that selectively target B-ALL via ligand-directed affinity. We hypothesize that nanoscale combination therapies developed using this approach will improve both cell selectivity and malignant cell killing and extend easily to additional drug combinations and cancers.

60. Epidemiology of Maternal and Child Hepatitis C Virus Infection in Ohio

Kennedy, Samuel; Gowda, Charitha; and Honegger, Jonathan

Chronic Hepatitis C infections lead to severe liver damage and place tremendous burdens on healthcare systems. Recently, several investigations have noted a rise in Hepatitis C virus (HCV) infections in the Appalachian region of the United States, especially among pregnant mothers, warranting further study. It is believed that current methods underestimate the true HCV infection rate. Therefore, this paper designed a method to optimally predict and characterize HCV infection among delivering mothers by linking two databases: Vital Statistics (VS) and the Ohio Disease Reporting System (ODRS). The two databases, which individually report Hepatitis C infection, were combined by matching entries where the same patient appeared in both records and those only captured by one source were included. The prospective method found that the incidence of HCV infection among delivering mothers in Ohio rose from 0.82% in 2012 to 1.53% in 2015, representing an increase in the estimated annual cases as compared to common methods that only use VS. This process offered more precise analysis of important demographic and clinical features of the study population by identifying women not present in VS that had differences in their marital status ($p=0.003$), race ($p<0.001$), county of residence ($p<0.001$), cigarette usage ($p<0.001$) and rate of diabetes ($p<0.001$). The most common HCV genotype discovered through this method was HCV-1 (73.6% - 76.6% of cases), and the highest rates of infection were discovered in rural, southern counties. This method can be used in future studies to more accurately monitor infection rates and population characteristics of Hepatitis C and other diseases.

61. Assessing Adherence to Hydroxyurea Suspension and Rates of Hospitalization in Pediatric Patients with Sickle Cell Disease

Kinsey, Joshua; and Gonzalez, Renzo

INTRODUCTION: The role of hydroxyurea (HU) in decreasing complications associated with sickle cell disease (SCD) has been well established in clinical trials. However, few studies show the role of adherence with improved outcomes. The primary purpose of this study was to assess the relationship of adherence to HU and rates of hospitalization. The secondary objective was to assess possible causes of non-adherence.

METHODS: Designed as a retrospective, cross-sectional study, participants of this study completed a voluntary survey to assess general adherence behaviors and any recent hospitalizations. Patients were divided into two groups (adherent and non-adherent) based on proportion of days covered (PDC) calculation. Results from the survey were also used to assess the occurrence of hospitalizations and evaluate the secondary endpoint.

RESULTS: There were N=103 total patients enrolled in this study with 50 patients in the adherent arm (defined as PDC \geq 80%) and 53 patients in the non-adherent arm (defined as PDC <80%). In the adherent arm, only 26% reported hospitalization in the past six months while 54.7% of patients in the non-adherent arm reported the same. When comparing the PDC based on whether patients reported “yes” for past hospitalization or “no”, there was a difference of -11.62 in PDC which was statistically significant (p=0.0016).

CONCLUSION: Adherence to HU (PDC \geq 80%) is associated with significantly lower rates of hospitalizations compared to non-adherence (PDC <80%). A collaboration between the involved community pharmacy and providers that prescribe HU to pediatric patients will begin in Fall 2018. Additional information will be collected on reported hospitalizations and additional analysis will be completed on all data collected.

TRANSLATIONAL IMPACT: Patients who were adherent to HU therapy reported hospitalizations 25% of the time; while patients who were non-adherent reported hospitalizations 56% of the time. Healthcare providers should encourage patients to remain adherent to HU therapy to decrease hospitalizations.

62. SERINC5 Induces Structural Reorganization of HIV-1 Env Glycoprotein Leading to Spontaneous Loss of Env Function

Kirschman, Jung Hwa; Marin, Mariana; Stano, Armando; Zwick, Michael; and Melikian, Gregory

SERINC5 incorporates into HIV-1 virions and inhibits virus fusion with target cells, and this effect is counteracted by Nef. Different Envs exhibit a broad range of sensitivities to SERINC5. The resistance to this restriction factor has been mapped to the variable loops V1-V3 of gp120 at the apex of Env trimer. We have previously shown that, in the absence of Nef, SERINC5 accelerates spontaneous functional inactivation of sensitive Envs, potentiates the neutralizing activity of anti-

gp41 antibodies and, ultimately, inhibits the formation of small Env-mediated fusion pores. Here, we attempted to elucidate a link between SERINC5 sensitivity and functional inactivation of Envs. Toward this goal, we measured the rates of functional inactivation of a panel of HIV-1 Envs consisting of sensitive lab adapted strains, as well as resistant primary isolates and transmitted/founder viruses. We found reasonable correlation between HIV-1 sensitivity to SERINC5, as measured by fold-reduction in infectivity, and the extent to which this factor accelerated Env inactivation (measured as reduction in T50). The effect of SERINC5 on the rate of inactivation of sensitive Envs was more profound than on resistant strains, while the unrelated VSV-G inactivation was not considerably affected. Increasing the density of Env in the virions did not noticeably affect SERINC5 incorporation, but rendered viruses more resistant to restriction and slowed down the Env inactivation rate. We also found that SERINC5 sensitized sensitive, but not resistant Envs strains to inactivation by a combination of a chaotropic agent and cold. These results support the model that SERINC5 drives structural reorganization of Env and thereby favors its inactivation. Experiments are underway to delineate the nature of conformational changes in Env induced by SERINC5 incorporation.

63. Association Between One-Carbon Metabolism Indices and DNA Methylation Status in Maternal and Cord Blood

Knight, Anna K; Park, Hea Jin; Hausman, Dorothy B; Fleming, Jennifer M; Bland, Victoria L; Rosa, Gisselle; Caudill, Marie A; Malysheva, Olga; Kauwell, Gail PA; Sokolow, Andrew; Fisher, Susan; Smith, Alicia K; and Bailey, Lynn B

One-carbon metabolism is essential for multiple cellular processes and can be assessed by the concentration of folate metabolites in the blood. One-carbon metabolites serve as methyl donors that are required for epigenetic regulation. Deficiencies in these metabolites are associated with a variety of poor health outcomes, including adverse pregnancy complications. DNA methylation is known to vary with one-carbon metabolite concentration, and therefore may modulate the risk of adverse pregnancy outcomes. This study addresses changes in one-carbon indices over pregnancy and the relationship between maternal and child DNA methylation and metabolite concentrations by leveraging data from 24 mother-infant dyads. Five of the 13 metabolites measured from maternal blood and 993 CpG sites changed over the course of pregnancy. In dyads, maternal and fetal one-carbon concentrations were highly correlated, both early in pregnancy and at delivery. The 993 CpG sites that changed over pregnancy in maternal blood were also investigated for associations with metabolite concentrations in infant blood at delivery, where five CpG sites were associated with the concentration of at least one metabolite. Identification of CpG sites that change over pregnancy may result in better characterization of genes and pathways involved in maintaining a healthy, term pregnancy.

64. Multimodal Virtual Reality Pediatric Assessment of Concussion: Pediatric DETECT

Kosoris, Nicole; Swanson, Erik; Liu, Brian; Medda, Alessio; Espinoza, Tamara; Gore, Russell; Wright, David; and LaPlaca, Michelle

Mild traumatic brain injury (mTBI) or concussion is a major medical and socioeconomic burden in the US and around the world. Injuries to children are a particular concern due to critical development periods and the potential loss of productive years. While an estimated 250,000 children (19 years old or younger) are treated in U.S. emergency departments for concussion per year, the actual incidence of youth concussion is likely much higher due to visits to non-ED physician and unreported and underdiagnosed instances. There is an urgent need for an objective and reliable concussion assessment tool in pediatric populations that considers the multitude of potential deficits associated with concussion. Unfortunately, there is not a single tool in use that tests the multiple expressions of concussion impairment, and delivers an objective assessment in the acute period following a suspected concussion. We have developed a pediatric version of the multimodal assessment tool, DETECT (Display Enhanced Testing for Cognitive Impairment and mTBI), for ages 9-12 that engages children for a series of carnival game-like virtual reality (VR) tests (Child DETECT). Child DETECT incorporates the key pillars of concussion assessment, neuropsychological (word recall, processing speed, conditional choice, reaction time), balance (nonpostural), and oculomotor (target tracking) testing. We used a Unity programming platform and implement the tests on an Android/Samsung VR Gear system. Testing of nonconcussed children in the target population is underway to assess usability and test-retest reliability. Use of VR in pediatric medicine is novel and may expand the possibilities for neurological assessment across a range of disorders. We anticipate that an animated, shortened battery of tests will not only engage children, but will ultimately provide an objective tool for improved clinical management.

65. Characterization of Super High Hospital Utilizers in a Population-based Cohort of Pediatric Sickle Cell Disease Patients, 2014-2017

Lai, Kristina; Ibrahim, Amenah; and Lane, Peter A

BACKGROUND: Disease severity and healthcare utilization varies widely among sickle cell disease (SCD) patients. From 2014 to 2017, CHOA admission rates for acute illness have increased despite efforts to improve outcomes. This analysis seeks to characterize SCD patients with the highest numbers of hospitalizations in order to understand trends in super high hospital utilizers (SHHU).

METHODS: The CHOA SCD database was reviewed for all patient admissions from 2014 to 2017. Patients with prior bone marrow transplant and admissions for elective procedures were excluded. Patients with 11 or more admissions in a given year were classified as SHHU and all records were linked to a treatment history database. Data were analyzed by SCD genotype, age at admission, gender, length of stay, discharge diagnosis, and treatment history. SHHU patients were compared with all SCD patients within the same age range.

RESULTS: We identified 21 SHHU (age 7+ years) with 447 admissions. Of all SCD patients in this age group, there were 1692 patients with 6088 admissions. SCD genotypes of SHHU were 63.9% HbSS, 22.2% HbSC, and 13.9% HbS β + thalassemia. The median age at admission was 16 years (IQR 14-17) and the majority (61.9%) were male. There were significant differences between SHHU and their peers, including history of treatment with hydroxyurea ($p < 0.001$) or chronic blood transfusions ($p < 0.02$), and proportion of admissions for pain crises (94.4% vs 77.0%, $p < 0.001$). Other characteristics were not significant. From 2014 to 2017, the total number of patients ages 7+ decreased, however the total number of admissions and the annual admission rate for ages 7+ increased. Prior to 2015, we did not identify any SHHU.

CONCLUSION: The unexpected increase in hospitalizations is likely attributable to the appearance and growth of SHHU. Genotype was not significantly different between SHHU and all patients of similar age, indicating that SCD genotype is not the strongest factor in determining progression to SHHU. This analysis highlights the need to further characterize and address inadequately managed pain in these patients. Future research should investigate the predictors of SHHU and trends in rising hospitalization rates in this SCD patient population.

66. Optical Bedside Monitoring of Cerebral Blood Flow in Children with Sickle Cell Disease during Head-of-Bed Manipulation

Lee, Seung Yup; Sanders, Bharat; Lam, Wilbur A.; and Buckley, Erin M.

Sickle cell disease (SCD) is an inherited blood disorder affecting millions of people worldwide and ~300,000 neonates each year. SCD has profound effects on the brain and the risk of stroke in children with SCD is 250 times greater than the general population. Without treatment, ~10% of patients will experience an overt stroke by age 10 and ~40% will have silent infarcts by age 15, which are clinically asymptomatic, but associated with worse neurocognitive outcomes.

Transcranial Doppler Ultrasound (TCD) is the standard screening tool for stroke risk in SCD children. However, TCD velocities are poor predictors of silent cerebral infarcts and they also have a low positive predictive value (PPV), leading to unnecessary transfusion. Thus, supplementary tools to TCD are needed for improved specificity in stroke screening and for optimization of therapeutic strategies. Microvascular, tissue-level cerebral blood flow (CBF) measurement holds promise as a complementary data to TCD measures of macrovascular velocities.

The goal of our study is to demonstrate the feasibility of a novel, non-invasive optical technique, known as Diffuse Correlation Spectroscopy (DCS), that can assess microvascular cerebral blood flow (CBF) in pediatric patients with sickle cell disease. In our study, we continuously measured frontal CBF during head-of-bed (HOB) manipulations in 22 children with sickle cell disease and 7 healthy controls. DCS-measured relative change in CBF (rCBF) during HOB lowering from 70° to flat was significantly lower in SCD patients than age-matched controls. Within the SCD patient group, we observed a wide range of CBF response to postural manipulation. Several subjects even exhibited paradoxical decreases in CBF with decreasing HOB angle. These results suggest that SCD patients may have impaired cerebrovascular autoregulation and thus limited ability to regulate CBF

in response to postural manipulation unlike healthy controls. Our study demonstrates the potential of our novel DCS technique as a non-invasive, bedside and continuous monitor of CBF and cerebrovascular autoregulation in children with sickle cell disease.

67. Generating Neutralizing Antibody Responses Using Recombinant Infectious Rhinovirus

Lee, Sujin; Tang, Roderick; Currier, Michael; and Moore, Martin

Human Rhinovirus (RV), a leading causative agent for common colds and the subsequent development of pneumonia, can exacerbate both asthma and chronic obstructive pulmonary disease (COPD). There is no vaccine against RV yet. As previous studies demonstrated that RV-neutralizing antibodies (nAb) correlates with protection, eliciting robust nAb responses might be a key factor for ideal RV vaccine development. Five neutralizing immunogenic (NIm) sites were identified. NIm-IA, IB, and IV are located on VP1, while NIm-II and III are located on VP2 and VP3, respectively. Since the capsid proteins (VP1, 2, 3 and 4) seem to be immunogenic, we hypothesize that a chimeric RV expressing the capsid proteins is sufficient to elicit nAb responses. The HRV-A76 insert expressing the four capsid proteins and 2A protein was cloned into a bacterial artificial chromosome (BAC) harboring cDNA expressing the full-length genome of HRV-A33. Utilizing reverse genetics, a capsid-chimeric recombinant RV strain, BAC HRV33cap76, was generated using the four capsid proteins and 2A protein from HRV-A76, and the remaining part of the genome from HRV-A33. Following RNA transfection and recovery, we found that the titer of BAC HRV33cap76 was similar to biological HRV33 or HRV76. We next immunized BALB/c mice intramuscularly with inactivated BAC HRV33cap76 mixed with alum adjuvant to determine its immunogenicity. The BAC HRV33cap76 immunization resulted in nAb against only HRV-A76. Our findings demonstrated that the capsid proteins are responsible for the generation of nAb against RV. As a capsid-chimeric recombinant RV was capable of eliciting robust nAb, it could be a novel vaccine platform to generate highly effective and safe vaccine for preventing RV infection.

68. Ultrathin, Comfortable, Multifunctional Biopatch for Safe and Effective Care of Neonatal and Infant Health Conditions

Lee, Yongkuk; Kim, Yun-Soung; Maher, Kevin; and Yeo, Woon-Hong

Even with fast technology development, neonatal and infant health care still lacks significant improvements over a decade. Approximately 30,000 children undergo open heart surgery each year and are discharged home following days to weeks of heart and respiratory monitoring in the hospital. Then, there is no available monitoring system when they are sent home because the existing systems for them still require lab-based testing machines and bench-top, non-portable systems. Even, recently developed wearable devices will not work since they are heavy, rigid, and bulky, which is far from ideal devices for them to use in a daily basis. In addition, these devices just use a fabric strap, rubber band, or an adhesive to cover and wear the rigid electronics on the body, which disrupts high-quality data recording and causes huge discomfort.

Here, we introduce an ultrathin, soft material-enabled, “skin-like” biopatch that addresses the technical barriers that currently limit our ability to offer comfortable, tissue-friendly sensing environment on the skin, along with multifunctional, wireless health monitoring. Specifically, we developed a nanomembrane, stretchable circuit platform, embedded in a soft membrane that can incorporate miniaturized commercial chip components via newly developed techniques: material transfer printing and hard-soft material integration. A working prototype device would represent the first demonstration of a tissue-friendly, stretchable, nanomembrane electronics that enables a long-range wireless, real-time monitoring of multi-physiological signals on babies, which will offer significantly improved safe and effective care for neonates and infants.

69. Differential Effects of Loss of the ASBT and OST alpha-beta on the Bicarbonate-rich Hypercholeresis Induced by NorUrsodeoxycholic acid

Li, Jianing; Rao, Anuradha; Pachura, Kimberly; Wynn, Grace; Ferree, Courtney; Daniela Fuchs, Claudia; Trauner, Michael; Dawson, Paul.

BACKGROUND: 24-norursodeoxycholic acid (norUDCA) has shown greater benefit for treatment of liver disease than ursodeoxycholic acid (UDCA) in preclinical animal studies and in clinical trials. The superior efficacy of norUDCA versus UDCA has been attributed to enhanced cycling between the biliary tract and liver (cholehepatic shunting). However, the role of the bile acid transporters versus passive diffusion in the absorption and cholehepatic shunting of norUDCA has not been fully explored. The primary aims of this study was to determine if an intact enterohepatic circulation and the major bile acid transporters, ASBT and OST α -OST β , are required for the therapeutic actions of norUDCA.

METHODS: Transport of UDCA and norUDCA were evaluated in ASBT-expressing MDCK cells. Male WT, Asbt KO, or Ost α KO mice (C57Bl/6J; 13 weeks of age) were fed with chow diet, or chow diet supplemented with norUDCA (0.5% w/w) for one week, and then used to measure bile flow rate, biliary bicarbonate secretion rate, bile pH and concentration of other biliary solutes.

RESULTS: UDCA inhibited [3 H]taurocholate uptake by human ASBT in a dose-dependent fashion, whereas norUDCA had no inhibitory activity over the same concentration range. Feeding chow plus 0.5% norUDCA for 7 days significantly increased bile flow rate (5- to 6-fold), biliary bicarbonate concentration (~1.5- to 2-fold), and biliary bicarbonate output (9- to 12-fold) in both WT and Asbt KO mice. By contrast, the biliary bicarbonate concentration was not increased in norUDCA-fed Ost α KO. The increases in bile flow and biliary bicarbonate output associated with norUDCA feeding observed in WT mice were attenuated in Ost α KO mice.

CONCLUSIONS: The finding that loss of OST α -OST β attenuated the biliary bicarbonate output induced by norUDCA may reflect a direct role of OST α -OST β in norUDCA transport or may be secondary to adaptive changes in Ost α KO mice. The results support the concept that the ASBT is not directly involved in the absorption, cholehepatic shunting or actions induced by norUDCA. The results also provide support for further investigation of the therapeutic potential of a combination of norUDCA and an ASBT inhibitor for forms of cholestatic liver disease.

70. Detection of Helicobacter pylori and Clarithromycin Resistance by PCR

MacDonald, Heather; Roberts, Leah; Caltharp, Shelley; Rogers, Beverly; and Jerris, Robert

Persistent Helicobacter pylori (HP) infection in pediatric patients due to antimicrobial resistance is increasing and is of concern as a risk factor for developing gastric cancer. Treatment with a PPI and dual antimicrobial therapy including clarithromycin (C) is routine. However, antimicrobial resistance detection is difficult as so few laboratories are able to cultivate or perform susceptibility testing on this fastidious organism. We have co-developed an assay with DiaSorin Molecular to detect the organism and mutations in the 23S rRNA gene responsible for C resistance (R). This pilot study using a 2 hour PCR assay and melt curve analysis, established the limit of detection for both the organism and the resistant genes to be 10 cells. In blinded studies using reference strains from the Centers for Disease Control (CDC), we accurately detected resistance or susceptibility in 33 of 34 isolates (16 C R, 18 C susceptible). The minimum inhibitory concentration for C R strains ranged from 2-8 mcg/mL. One isolate that tested as C R by CDC failed to be detected in the assay.

In addition, we tested 3 formalin-fixed paraffin embedded tissues, positive by histopathology for HP. No culture was available for antimicrobial profile (which is the norm for the majority of cases). Sections were deparaffinized and nucleic acid was extracted and run in the assay. The assay detected HP in each of the tissues with no R to C.

This assay holds promise to become a commercially available assay to rapidly detect HP and C R.

71. Analyzing Correlations Between Population-level Socioeconomic Markers and Patient-level Clinical Risk Factors for Adverse Pediatric Surgery Outcome

Mahajan, Ruhi; Shin, Eun; Shaban-Nejad, Arash; Davis, Robert L; Akbilgic, Oguz

In comparison with white children, black children are more than twice as likely to die after surgery, have higher rates of post-surgical morbidity, and significantly higher hospital charges. Most studies on the disparities in pediatric surgery outcomes have focused on patient-level Clinical Risk Factors (CRFs) or population-level Societal, Economic, and Environmental Factors (SEEFs) in isolation, without considering the interactions and correlations between the two.

To understand the underlying mechanisms leading to racial disparities in pediatric surgery outcome, we integrated patient-level preoperative CRFs and surgery outcomes with population-level SEEFs. We used a dataset from a cohort of 436 patients who underwent surgery at Le Bonheur Children's Hospital (LBCH) during 2014-17 from 29 zip-codes in Shelby County, Memphis, TN. The patient level data was a part of LBCH subset of National Surgery Quality Improvement Program-Pediatrics dataset. Population-level data were collected from the 2010 United States census data and 2015 housing-neighborhood quality survey data from Memphis Property Hub. We conducted a correlation analysis of 24 CRFs, 9 SEEFs, and 8 surgical outcomes and thereby tested the significance of correlations using a two-tailed t-test.

Our results suggest a positive correlation ($p < 0.05$) between children requiring supplemental oxygen support at the time of surgery with SEEFs, such as blight prevalence, trash presence, and percentage of the individuals living below the poverty line in the neighborhood. Further, the need of oxygen support showed a significant correlation with three surgical outcomes—postoperative wound disruption, unplanned intubation, and transfusion. An association of abovementioned SEEFs ($p < 0.05$) and surgical outcomes ($p < 0.01$) was also found in children with the hematologic disorder. The presence of minor cardiac risk factor in pediatric patients showed correlation ($p < 0.05$) with SEEFs, such as trash presence and rate of individuals living below the federal poverty line in the neighborhood. These patients were found to be at a higher risk of postoperative wound disruption ($p < 0.01$), unplanned intubation ($p < 0.01$), transfusion ($p < 0.05$), and sepsis ($p < 0.05$).

The correlations between population-level SEEFs and patient-level CRFs suggest further investigation of underlying causality patterns. Such knowledge can facilitate design, implementation, and evaluation of targeted interventions to address disparities in pediatric surgery outcome.

72. The Adaptive Value of Attending to Social Stimuli Differs for Toddlers with Autism Spectrum Disorder and Williams Syndrome

Markert, Sarah; Olmstead, Jack; Ford, Aiden; Klin, Ami; Klaiman, Cheryl; Lense, Miriam; Jones, Warren; and Shultz, Sarah

BACKGROUND: Throughout the first years of life, infants filter environmental information by attending to what they find most important, creating a unique subjective experience that shapes their developing cognition. Both children with Autism Spectrum Disorder (ASD) and Williams Syndrome (WS) exhibit intellectual, linguistic, and social disability, but with distinct phenotypic profiles emerging by the second year of life (reduced vs. heightened interest in the social world). By identifying disorder-specific patterns of social visual engagement, this study aims to gain insight into the distinct developmental processes underlying the presentation of these neurodevelopmental disorders.

OBJECTIVE: To investigate: (1) clinical profiles and patterns of visual fixation to social stimuli in children with ASD, WS, and TD controls and (2) whether and to what extent fixation patterns are related to communicative competence.

METHOD: Eye-tracking data were collected while 47 toddlers (18 ASD, 11 WS, and 18 TD) watched video scenes of an actress caregiver engaging in child-directed communication. Visual fixation was quantified as the percentage of time spent looking at 4 regions of interest (eyes, mouth, body, object). Between-group comparisons of assessment scores on the Autism Diagnostic Observation Schedule (ADOS) and the Mullen Scales of Early Learning were made, and within-group analyses tested for correlations between assessment scores and percentage fixation to each region of interest.

RESULTS: For children with WS and TD controls, percent fixation on the mouth was positively correlated with expressive and receptive language scores (all p's <0.05). By contrast, mouth-looking was not correlated with either expressive or receptive language for children with ASD (all p's >0.2).

CONCLUSIONS: This research reveals a disorder-specific developmental process that may contribute to the social and cognitive phenotype of ASD. Visual engagement with the mouths of others does not share the same adaptive value for children with ASD, who show no association between mouth-looking and language scores. This suggests that toddlers with ASD may seek and attend to the mouths of others for very different reasons than do TD and WS children, and subsequently likely yield different learning experiences and expertise that may contribute to their behavioral and language outcomes.

73. The Anti-Tumoral Role of Macrophages in a Murine Sonic Hedgehog Medulloblastoma Model

Maximov, Victor; Chen, Zhihong; Wei, Yun; Robinson, M. Hope; Shanmugam, Nithya S.; Hambardzumyan, Dolores; and Kenney, Anna M.

Medulloblastoma (MB), the most common malignant pediatric brain tumor, has a 70% survival rate but standard treatments often lead to devastating life-long side effects, and recurrence is fatal. MB was classified based on molecular and genetic profiles and resulted in four distinct subgroups. One subclass of MB is marked by activation of Sonic hedgehog (SHH) pathway components and targets, and accounts for approximately 30% of cases. This class has been successfully modeled in vivo in murine models, which closely recapitulate human disease, providing a convenient and relevant model system for analyzing the SHH MB subclass in vivo. To better understand the mechanisms of tumor growth and recurrence, recent attention has been focused on determining the composition and role of non-tumor cells comprising the tumor microenvironment (TME). Tumor-associated macrophages (TAMs) were recently shown to have a pro-tumoral role in glioblastoma tumors, but the role of TAMs in MB is still to be determined. Recently, it was reported that of the subgroups, human SHH MB has the greatest number of TAMs, as well as increased expression of macrophage-associated genes. Here, we demonstrate a functional role of TAMs in both in vitro and in vivo models. We show that reduction of macrophage numbers accelerates animal mortality in a murine model of SHH MB, and that increased levels of macrophage inflammatory markers correlate with improved survival in patients, indicating that in contrast to their role in gliomas, macrophages in SHH MB are beneficial. Further investigation of the TME and TAMs in MB has the potential to elucidate immune system involvement in the disease and lead to development of novel treatment options harnessing the patients' own immune system.

74. Formulation and Evaluation of RSV Virus-Like Particle Microparticulate Vaccines for RSV Infection

Menon, Ipshita; D'Sa, Sucheta; Kang, Sang-Moo; and D'Souza, Martin

INTRODUCTION: The respiratory syncytial virus (RSV) is highly prevalent in children and manifests itself in the form of bronchiolitis and pneumonia. One of the major proteins present in the virus, is the fusion protein F, which can be integrated into a virus-like particle (VLP), yielding a highly immunogenic F-VLP antigen.

METHODS: In this study, the F-VLP antigen was incorporated into a biodegradable polymer matrix and its in vitro immunogenicity was evaluated in a mechanistic study to evaluate surface co-stimulatory expression, wherein antigen presenting cells were stimulated with the vaccine-adjuvant combinations. Further, vaccine-adjuvant combination was administered to C57BL/6 mice via the transdermal route using microneedles (AdminPatch®) to evaluate the immunogenicity of the vaccine in vivo.

RESULTS: Particulate vaccines with or without adjuvants significantly increase expression of immune markers such as nitric oxide and resulted in enhanced cell-surface expression of CD80/86, CD40, MHC II and CD54/ICAM-I on dendritic cells. In vivo studies using the non-invasive transdermal route demonstrated elevated humoral and cell-mediated immune responses in a mouse model.

CONCLUSION: These preliminary studies prove the efficacy of the RSV F-VLP microparticulate vaccine as a novel immunotherapeutic strategy in the future development of a vaccine against RSV.

TRANSLATIONAL IMPACT: RSV leads to the hospitalization of approximately 3.4 million children annually and nearly 160,000 people die due to RSV infection worldwide. There are no licensed vaccines available today for RSV. The successful development of this vaccine would open a new paradigm in the treatment of RSV and would in turn save many human lives.

75. The Effects of Prospectively Following High-Risk Infant Siblings with ASD: Observation Positively Alters Outcome

Micheletti, Megan; Jones, Warren; Shultz, Sarah; Klin, Ami; Hoffenberg, Sara; Saulnier, Celine; and Klaiman, Cheryl

BACKGROUND: The past 10 years have seen an increase in prospective studies of the infant siblings of children with autism spectrum disorder (ASD), whose risk of also having the condition is approximately 20%. Direct observation of this population from the first months and years of life provides a unique window into the early emergence of ASD. No study to date, however, has examined how this early developmental surveillance, in and of itself, may impact the course of a child's development.

OBJECTIVE: Measure the effects of prospectively following infants later diagnosed with ASD on parent-reported first concerns, clinical presentation, and service utilization.

METHODS: Children were selected to form two groups: 1) a prospectively-followed cohort (children who completed a prospective study including 10 visits from birth to 36 months); and 2) a

community-referred cohort (children referred for clinical assessment at a single timepoint). All children in each cohort: 1) received a clinical best-estimate diagnosis of ASD at 24-36 months; 2) had an older sibling diagnosed with ASD; and 3) had completed measures of child demographics, development, and service utilization. Cohorts were then matched on sex, race, cognitive ability, maternal age, maternal history of infertility, pregnancy complications, and gestational age to yield cohorts of n=18 prospectively-followed and n=18 community-referred.

RESULTS: Age at parent-reported first concern was significantly earlier in the prospectively-followed cohort (11.7 months) than the community-referred cohort (15.5 months), $p=0.039$. The prospectively-followed cohort had lower ADOS severity scores ($p=0.013$), and higher receptive ($p=0.011$) and expressive ($p=0.027$) language abilities relative to the community-referred cohort. Although there were no differences in age at therapy onset, prospectively-followed children were more often enrolled in comprehensive early intervention ($p=0.026$).

DISCUSSION: Compared to a matched community-referred cohort, prospectively-followed children had earlier parent concerns, higher enrollment into early intervention, and less severe ASD symptoms. These differences were observed despite similarities in demographics, cognitive levels, and parents' experience with a child on the spectrum. Developmental surveillance may be a framework to aid parents in identifying ASD and accessing early intervention, likely to have profound positive cascading effects on child development.

76. Incidence and Predictors of Respiratory Adverse Events during Induction Therapy in Children with Acute Myeloid Leukemia

Miller, Lane; Castellino, Sharon; Mertens, Ann; Keller, Frank; and Woods, William G.

BACKGROUND: Survival in childhood acute myeloid leukemia (AML) has plateaued at 60-70%, with induction death occurring in 4-11% of patients. While pulmonary complications are known to contribute to pediatric AML induction morbidity and mortality, our understanding of the incidence, categories, and risk factors for respiratory adverse events (AEs) is incomplete.

OBJECTIVES: To estimate the incidence of respiratory AEs occurring during induction therapy for pediatric AML, categorize and grade these AEs, and identify risk factors for AE development.

METHODS: Using manual chart abstraction, we retrospectively followed a cohort of de novo pediatric AML patients (age \leq 21) from initial presentation through day 42 of induction chemotherapy. Outcomes included any NCI CTCAE grade 2-5 respiratory AE or death from another cause. Demographic, disease, and treatment-related data were abstracted. Descriptive statistics, survival analysis, bivariate analysis, and multivariable analysis were performed (SAS v9.4, Cary, NC).

RESULTS: Among 113 eligible subjects, 53.1% (n=60) experienced 74 grade 2-5 respiratory AEs. Mechanical ventilation was required in 23% of all respiratory AEs (n=17). Peaks in incidence occurred between days 0-7 and days 14-21. Induction death occurred in 4.4% (n=5). Fluid overload

at any time (aOR 47.6 [95% CI: 5.7-395.1]) and older age at diagnosis (aOR 1.12 [95% CI: 1.01-1.24]) were independently associated with AE occurrence. Positive fluid overload status (aHR 5.63 [95% CI: 3.42-9.29]), positive infection status (aHR 2.29 [95% CI: 1.30-4.02]), elevated initial WBC (aHR 1.003 [95% CI: 1.000-1.005]), and male gender (aHR 1.59 [95% CI: 1.05-2.38]) were independently associated with increased instantaneous risk for AE development.

CONCLUSION: We describe a higher incidence of respiratory AEs during childhood AML induction than previously described. Fluid overload at any time and older age at diagnosis are independently associated with AE development. Positive fluid overload status, positive infection status, elevated initial WBC, and male gender were independently associated with increased hazard for AE development. Interventions focused on fluid overload and infection prevention and management should be further addressed in this population to reduce early respiratory complications and prevent potential morbidity and mortality.

77. Using Laboratory Result Data From an Administrative Database to Describe Accurate Adverse Event Rates During Pediatric Leukemia Therapy

Miller, Tamara; Getz, Kelly; Jen, Emily; Huang Yuan-Shung; Li, Yimei; Bagatell, Rochelle; Seif Alix; Fisher, Brian; Przepiorka, Donna; and Aplenc, Richard

BACKGROUND: Adverse events (AE) on cooperative oncology group clinical trials are manually reported by research assistants using the National Cancer Institute Common Terminology Criteria (CTC). Our prior work demonstrated that pediatric oncology clinical trials markedly underreport laboratory AEs and that automated AE ascertainment using electronic health record (EHR) data at 1 hospital are as accurate as gold standard chart abstraction. This study sought to prove that laboratory data in the Pediatric Health Information System Plus (PHIS+) administrative database are consistent with EHR data and to use PHIS+ data to describe AE rates in Induction for pediatric acute myeloid and lymphoblastic leukemias (AML, ALL). Accurate estimates of laboratory AE rates are currently absent in the pediatric oncology literature.

METHODS: PHIS+ includes laboratory results submitted by 6 large children's hospitals from 2007 to 2012. EHR and PHIS+ laboratory dates, times, and results for 3 tests at 1 site were compared to assess PHIS+ accuracy. PHIS+ results were cleaned, graded and processed according to CTC v4 definitions for 12 AEs. AE rates were summarized by highest grade during Induction.

RESULTS: Laboratory data on 171 AML and 1099 ALL patients were extracted from chemotherapy start date to D+35. PHIS+ and EHR data at 1 hospital showed good concordance (Potassium: AML 97%, ALL 95.5%; Alanine Aminotransferase: AML 96.5%, ALL 82.2%; Absolute Neutrophil Count: AML 96.9%, ALL 91.2%). Missing PHIS+ data were from outpatient tests or admissions ending in 2012, the last year of PHIS+ data. The percentage of courses with a highest grade of each AE ranged from 0-28.7% for grade 3 and 0-14.1% for grade 4 chemistry results. At least 93% of AML and 65.2% of ALL courses had grade 3 or 4 hematologic AEs.

CONCLUSIONS: PHIS+ data are consistent with EHR data and can be used to determine accurate laboratory AE rates. These AE estimates can be used to counsel patients about anticipated AEs during Induction therapy and as an accurate baseline comparison for experimental therapies.

78. Natural Language Processing of Radiology Reports to Capture Adult Respiratory Distress Syndrome in Pediatric Leukemia

Miller, Tamara; Masino, Aaron; Yehya, Nadir; Burrows, Evanette; Grundmeier, Robert; and Aplenc, Richard

BACKGROUND: Pediatric cancer therapies cause substantial treatment-related side effects. Clinical trial adverse event (AE) reports inform clinicians and patients about potential therapy complications. Currently, cooperative oncology group trial AE reporting requires manual review of the medical record based on the National Cancer Institute Common Terminology Criteria. Despite this time-consuming process, AEs are globally under-reported on trials. Our prior work accurately ascertained laboratory-based AEs using automated extraction of electronic medical record (EMR) data. This study aimed to use natural language processing (NLP) to extract radiology report data from the EMR in order to ascertain a clinically significant, complex AE, adult respiratory distress syndrome (ARDS), in pediatric leukemia patients.

METHODS: This study used a cohort of 459 patients previously identified to have ARDS and a cohort of 339 patients with acute leukemia without ARDS. Chest x-ray (CXR) reports were processed using NLP to identify x-rays with bilateral infiltrates. A training set of 80% of all CXR reports was used to form a vocabulary representing all words used across all training documents except common English words using the Python Natural Language toolkit. Each CXR report was tokenized by division into individual words as a vector of numbers indicating which words from the vocabulary were present in the document. A grid search was performed over-tuning parameters for logistic regression, random forest, and support vector machines with a non-linear Gaussian kernel using 5-fold cross validation to select the best tuning parameters for identification of ARDS. The overall best model configurations were evaluated in the test set of the 20% of CXR reports not used in the training sample.

RESULTS: The training set included 367 patients with ARDS and 271 controls. The test set included 92 patients with ARDS and 68 controls. In the test set the accuracy was 95.6%, sensitivity was 96.7%, specificity was 94.1%, PPV was 95.7% and NPV was 95.5%.

CONCLUSIONS: NLP of radiology reports can be used as an effective screen for ARDS. This method will be further evaluated to prove that NLP can be used as a screening tool in a general cohort of leukemia patients with unknown ARDS status.

79. An Expression Based Risk Score for Prediction of Colectomy in Pediatric Ulcerative Colitis

Mo, Angela; Marigorta, Urko M.; Hyams, Jeffrey; Mack, David; Boyle, Brendan; Griffiths, Anne; LeLeiko,

Neal; Baldassano, Robert; Rosh, Joel; Keljo, David; Markowitz, James; Walters, Thomas; Kugathasan, Subra; Denson, Lee; and Gibson, Greg

BACKGROUND: Accurate early prediction of colectomy in pediatric ulcerative colitis (UC) would be valuable to guide therapeutic interventions. Current treatment schemes based on the Pediatric UC Activity Index (PUCAI) are based solely on clinical data and have limited predictive value. Incorporation of gene expression data may improve prognostic accuracy.

AIM: Examine the utility of gene expression data at diagnosis to predict whether children newly diagnosed with UC will progress to colectomy by 52 weeks following diagnosis.

METHODS: Children with newly diagnosed UC were initially treated with standardized regimens of mesalamine or corticosteroids (CS) based upon initial disease activity in the PROTECT Study: Predicting Response to Standardized Pediatric Colitis Therapy (5U01DK09574). RNA-Seq was performed on rectal biopsies from a subset of 211 newly diagnosed pediatric UC patients in the PROTECT cohort. We first performed differential expression analyses comparing gene expression between patients who experienced colectomy vs. those that did not undergo colectomy by 52 weeks. Then we performed principal component analysis (PCA) based on genes differentially expressed at $P < 0.01$ to derive a discriminative risk score based on principal components.

RESULTS: Of 211 sequenced individuals, 15 progressed to colectomy by week 52 and 196 did not. We detected 225 differentially expressed genes. A risk score based on principal component 2 of these genes more accurately distinguished colectomy outcome than PUCAI, initial treatment, or remission by week 4 ($p < 0.0001$ vs. $p = 0.0004$, $p = 0.0006$, and $p = 0.0003$ respectively). 16 genes showed strong loading with PC2, including sulfotransferases, cell membrane and matrix genes, and metabolism genes. Plotting the PC-derived risk score by PUCAI reveals that the majority of individuals who progress to colectomy cluster in the lower right quadrant, implying that a composite score may be most predictive of disease progression.

CONCLUSIONS: Our findings suggest that gene expression data at baseline can be utilized to develop risk scores for colectomy in pediatric UC. In combination with other metrics of disease severity, expression based risk scores may help to inform treatment decisions.

80. Identifying a Strong Candidate Variant for Short Stature and Insulin Resistance Using Whole Genome Sequencing

Mosley, Trenell; Kugathasan, Logan; Wang, Chuan-En; Wilcox, William; and Zwick, Michael

We identified two Middle Eastern adolescent sibling probands (a boy and a girl) presenting with short stature, particularly of the hands and feet, and insulin resistance. The probands are offspring of unaffected consanguineous parents. We hypothesize the phenotypes are the result of a novel autosomal recessive disorder and predict both probands should be homozygous for the same identical-by-descent allele. To identify candidate variants, we performed whole-genome sequencing and two orthogonal variant analyses: 1) a genome-wide search for rare, high-CADD (MAF < 0.001 ;

CADD < 15) variants homozygous in both cases, and 2) rare, high-CADD variants located in runs of homozygosity where both samples were homozygous for the same alleles. The analyses converged on nine candidate variants on chromosome 3. One variant in the DUSP7 gene (g.52050873:T>C; hg38) is rare in the general population (MAF=0) and is a tyrosine to cysteine missense mutation (p.Tyr401Cys). DUSP7 is a dual-specificity phosphatase that interacts with the MAP kinase (MAPK) pathway, mutations in which result in dwarfism. MAPK also interacts with the insulin pathway. DUSP7 is a highly constrained gene, and the mutated residue is evolutionarily conserved. We showed that the DUSP7 variant follows the expected segregation pattern: it is homozygous in the affected siblings, and heterozygous or absent in unaffected members of the pedigree. Collectively, this evidence suggests the DUSP7 variant is a strong candidate for the described disorder. Future steps include conducting molecular studies to determine if the observed mutation functionally or structurally disrupts the DUSP7 protein. Investigation of this putative causal variant will provide insight into the dynamics of dual-specificity phosphatases in developing chondrocytes and the insulin signaling pathway, while broadly elucidating the mechanisms influencing metabolic insulin signaling, bone development, and the etiology of insulin resistance.

81. Long-Term Prognostic Patterns In Pediatric Epilepsy Cohorts: Review Of Recent Literature.

Naik, Kushal B; McCallum, Susan; and DeGrauw, Ton J

OBJECTIVES: The aim of this paper is to provide a synoptical review of the evidence generated by recent longitudinal and retrospective cohort studies to assess the long-term prognostic patterns in pediatric epilepsy.

BACKGROUND: Epilepsy is a heterogeneous disease where certain types are self-limiting while others are intractable and may extend for several decades into adulthood. Several reports on the long-term prognosis of early-onset epilepsy have been published that suggest the wide range of possible outcomes and prognostic patterns. Some studies were hospital- or community-based while others were cross-sectional or from administrative datasets.

DESIGN: We reviewed 14 studies published between 2012 and 2017, of which 4 were from secondary or tertiary referral centers and the remaining were essentially population-based studies.

RESULTS: The population assessed by these studies was early-onset pediatric age group among which, 3 studies focused on infants. The mean follow-up was 16.6 years (Range: 2.0-27.7). Epilepsy prognosis was quite variable and early therapy or remissions were not predictive of the final outcomes in patients in the long term. Often, a relapsing-remitting pattern was observed on long-term follow-up. While etiology was one of the dominant predictors of outcome, it was difficult to establish other consistent predictors. Epilepsy may reappear in early-onset epilepsy patients after even a ten-year remission which, according to current definitions, is considered resolved epilepsy. Different prognostic patterns become evident in cohorts followed for several decades. Children with complicated epilepsy had higher mortality than those without complications. Definitions of terms

like early, late, long-term, terminal or permanent remission are likely to evolve as more evidence is generated.

CONCLUSION: The temporal aspects of remission and relapse associated with treatment in the long-term are extremely important and there is paucity of population-based data that can provide definitive answers about the long-term prognosis of each early-onset epilepsy syndrome. Pediatric patients need to be followed into adulthood, after the patients transition out of pediatric care to adult healthcare.

82. Protection Against Metabolic Changes Induced by a Western Diet by *Lactococcus lactis*

Naudin, Crystal; Maner-Smith, Kristal; Darby Trevor; Ortlund Eric; and Rheinallt Jones

Childhood obesity is linked to chronic inflammation and immune dysfunction which intensifies the development of co-morbidities such as cardiovascular disease, type 2 diabetes mellitus, and insulin resistance. To develop interventional therapies for obesity, we tested the efficacy of supplementing a newly identified strain of probiotic at reducing the effects of a high sugar and high fat western diet on female mice. Whereas a western diet elevated BMI, serum cholesterol, hepatic lipid content, supplementation of the western diet with a strain on *L. lactis* resulted in significantly lower BMI, lower serum cholesterol, lower hepatic liver content, and higher glucose tolerance. Assessment of the liver by lipidomic mass spectrometry revealed that administration of *L. lactis* altered the lipid profile within the liver. Together our data shows that this strain of *L. lactis* inhibits the negative influences of a western diet in the liver and propose that this probiotic may be used as a therapeutic intervention to treat obesity.

83. Specificity of Social Visual Engagement Patterns in Toddlers with Autism Spectrum Disorder and Williams Syndrome

Olmstead, Jack.; Markert, Sarah; Ford, Aiden; Klin, Ami; Jones, Warren; Lense, Miriam; and Shultz, Sarah

The social phenotypes of Autism Spectrum Disorder (ASD) and Williams Syndrome (WS) are often contrasted with one another (Bellugi, 2001). In general, individuals with ASD exhibit reduced social interest, whereas those with WS are atypically prosocial and initiating. The present study seeks to investigate patterns of social visual engagement in groups of toddlers with ASD or WS. Twenty-four-month-old children were eye-tracked while viewing video scenes of naturalistic caregiver interactions. Percentage of fixation time during viewing on 4 regions of interest (eyes, mouth, body, and object) were calculated for each participant. Toddlers with WS fixated significantly more on eyes compared to the ASD, developmentally delayed (DD), and typically developing (TD) groups (all p 's < 0.01). By contrast, toddlers with ASD exhibited a trend toward less time spent looking at eyes compared to all other groups ($p=0.094$). Toddlers with WS also fixated significantly less on the mouth region relative to all other groups (all p 's < 0.01). Toddlers with ASD showed a trend towards increased fixation on the body region relative to TD toddlers ($p=0.057$). Finally, both ASD and WS groups spent more time looking at objects relative to TD toddlers (both p 's < 0.05). These data from

ASD and WS toddlers reveal marked and group-specific deviations from the fixation patterns observed in typical development. Toddlers with WS display a striking preference for looking at the eyes of others, whereas toddlers with ASD trend towards less eye-looking. In short, although toddlers with ASD share aspects of social and intellectual disability with WS and DD cohorts, respectively (see clinical characterization scores), our results reveal the specificity of reduced eye-looking to the social phenotype of ASD, as well as aspects of atypical social visual engagement—such as increased attention to objects—that may be common to developmental delays characterized by social disability. These results highlight distinct mechanisms by which developmental outcomes precipitate in ASD and Williams Syndrome.

84. Using a Mobile App to Collect Data for an International Pediatric Perioperative Outcomes Study: Feasibility Assessment

O'Reilly-Shah, Vikas; and Yoo, Young Moo (Daniel)

INTRODUCTION: The Lancet Commission on Global Surgery has established that 5 billion people worldwide do not have access to safe and timely surgical and anesthetic care and has called for a substantial increase in the access to care by 2030. "Anesthesiologist" is a free anesthesia calculator app for Android used globally. After establishing the demographic characteristics of the user base, we became interested in whether it would be feasible to recruit a global set of collaborators to undertake a prospective study of pediatric perioperative outcomes to support the goals of the Global Surgery 2030 initiative.

METHODS: As previously described, we collected survey and analytics data from the users of "Anesthesiologist". This data included provider role, location information, and the type of information being accessed in the app. Data included in the present analysis was collected from December 2015 through September 2017. Participants were asked two survey questions: one assessing their level of interest in collaborating on a perioperative outcomes study, and one asking for their contact information.

RESULTS: Of 20,749 consented study participants who used the app since deploying the survey, 3,398 (16.3%) answered the question regarding level of interest. The distribution of interest is shown in. The distribution of World Bank country income levels associated with these responses is shown. Contact information was obtained from 1,315 respondents, and the number of users per country providing contact information is illustrated.

CONCLUSIONS: This mobile application provided a reasonable platform for initial contact with potential collaborators in a multinational study of pediatric perioperative outcomes. Such studies have recently been performed in adult populations, and collection of such data is consistent with the goals of the Lancet Commission on Global Surgery. This work builds on previous findings of feasible data collection via smartphone by extending it to collaborator identification. As the project continues to mature, we will assess the rate at which this interest translates to participation in the planned study.

85. When Are We Operating on Kids? A Potentially Simple Intervention to Improve Outcomes in Developing Countries.

O'Reilly-Shah, Vikas; Easton, George; and Gillespie, Scott

INTRODUCTION: According to the Lancet Commission on Global Surgery, over 5 billion people have deficient access to basic surgical and anesthetic care. The rapid global adoption of mobile health (mHealth) smartphone apps by providers provides opportunities to study global medical practice patterns, track access to care, and disseminate best practice information. App analytics, combined with in-app demographic surveys, can provide powerful tools for the collection of this data.

METHODS: We studied users of a free anesthesia calculator app used in nearly every country in the world. We combined traditional app analytics with in-app surveys to collect user demographics and feedback. Chi-square tests were used for statistical comparison, using Holm's method to correct for multiple comparisons.

RESULTS: Mining data on ~617k patient age entries from 48,034 subjects in 212 countries, we found most app uses were associated with the care of pediatric patients: ~147k (24%) of patient records were less than one month old, and ~465k (75%) were less than twelve years old. We observed significant differences in age of the patients for which the app was consulted as a function of country income level. Specifically, the proportion of neonates, infants, and toddlers was higher in lower income countries. We also observed significant differences in the hour of the day when the app was used; for neonates, infants, and toddlers, app uses were observed at a significantly higher rate in the evenings and at night in lower income countries. In particular, the app was consulted at a substantially higher rate for neonatal patients in lower-middle-income countries. Except for low vs lower-middle income in all categories, all pairwise comparisons were statistically significant at the 0.005 significance level.

CONCLUSIONS: Country income level appears to be an important predictor of the use of mHealth clinical decision support, which may suggest higher need for decision support in the care of this vulnerable population. There is evidence that nighttime procedures are associated with increased complication rates and reduced efficiency. The increased rate of evening and nighttime procedures in lower income countries is potentially a very easy target for intervention in improving outcomes.

86. Validation of a Screening Tool for Child Sex Trafficking Among Patients with High-Risk Chief Complaints in a Pediatric Emergency Department

Peckham, Sheri-Ann; Greenbaum, V. Jordan; Agarwal, Maneesha; McCracken, Courtney; Zmitrovich, April; Harper, Elizabeth; and Simon, Harold K.

OBJECTIVES: To apply and validate a screening tool to identify victims of child sex trafficking (CST) in a pediatric emergency department population.

METHODS: This prospective, observational study was conducted from 7/2017-11/2017 at the pediatric emergency department (PED) of a free-standing, inner-city children's hospital. Patients 10-18 years of age presenting with chief complaints related to high-risk social or sexual behaviors were recruited in a representative convenience sampling. A previously developed 6-item screening tool was administered verbally to participants. A patient was considered a "true" CST victim if any information obtained during the visit indicated that their circumstances fulfilled the federal definition of CST. Descriptive statistics were calculated for all variables of interest. CST screening tool analysis included sensitivity, specificity, and positive and negative predictive values (PPV, NPV). Those patients identified as positive for CST were referred to social services and received the current standard of care.

RESULTS: 254 patients met chief complaint screening criteria and eligibility; 215 were approached to participate; 203 agreed to participate. Of the 203 participants, 100 screened positive with the tool (49%). The total number of CST victims identified was 11, 10 of whom screened positive. With a cut-off score of 2 positive answers the tool demonstrated a 90.9% sensitivity, 53.1% specificity, 10.0% PPV, 99.0% NPV, 0.97% false negative rate, and 90.0% false positive rate.

CONCLUSIONS: Applied to an inner-city PED population of 203 participants with high-risk chief complaints, the screening tool has high sensitivity and high negative predictive value. This makes it appropriate for an initial screening to rule out CST in this high-risk population. The tool has a low specificity and PPV, so further evaluation of patients is required to confirm a positive screen. Applicability for broader use and additional practice settings are warranted given the significant positivity rate among those presenting with high-risk concerns.

87. Olecranon Fractures in Children and Adolescents: Outcomes Based on Fracture Fixation

Perkins, Crystal; Busch, Michael; Christino, Melissa; Axelrod, Jed; Devito, Dennis; Fabregas, Jorge; Flanagan, Jill; Murphy, Joshua; Olszewski, Dana; Schmitz, Michael; Schrader, Tim; and Willimon, S. Clifton

BACKGROUND: Open reduction and internal fixation with a tension band construct is the standard treatment for displaced transverse intra-articular olecranon fractures. The purpose of this study is to describe the outcomes of tension band fixation of olecranon fractures in children, specifically assessing the need for revision fixation and hardware removal

METHODS: Patients less than 18 years of age diagnosed with a displaced transverse intra-articular olecranon fracture and treated with tension band fixation between 2008 and 2017 were retrospectively enrolled. Operative treatment was with tension band wire (TBW) or tension band suture (TBS) constructs.

RESULTS: 46 patients, 36 males and 10 females with an average age of 12.3 years, were included. Surgical fixation was with TBW in 17 patients and TBS in 29 patients. Revision fixation due to failure and fracture displacement was required in 6% in the TBW group and 14% in the TBS group, $p=0.19$. The patients who required revision fixation in the TBS group were older (14.7 years vs 11.6

years, $p=0.05$) and heavier (70.5 kg vs 48.5 kg, $p=0.05$) than those in the same group who did not require revision fixation.

CONCLUSIONS: Pediatric olecranon fractures treated with TBW or TBS fixation unite in the majority of patients with similar need for hardware removal due to prominence and/or pain between fixation techniques. In a select group of older patients weighing greater than 50 kg, TBS constructs demonstrate increased failure rates, requiring revision fixation.

88. Allograft Augmentation of Hamstring Anterior Cruciate Ligament Autografts is Associated with Increased Graft Failure in Children and Adolescents

Perkins, Crystal; Busch, Michael; Christino, Melissa; Herzog, Mackenzie; Schaafsma, Belinda; and Willimon, S. Clifton

BACKGROUND: Anterior cruciate ligament (ACL) reconstruction in adolescents is commonly performed with hamstring autografts. In this population, with very high activity levels, graft rupture is the most common complication of ACL reconstruction. The purpose of this study was to evaluate the association of soft tissue graft constructs and graft rupture following pediatric and adolescent ACL reconstruction.

METHODS: A single-institution retrospective review was performed of consecutive pediatric patients 19 years of age and younger who underwent ACL reconstruction with transphyseal or anatomic drilling techniques and hamstring tendon autografts, with or without soft tissue allograft augmentation from 2012-2016. Graft constructs included 4-strand doubled semitendinosus and gracilis autograft (4-STG), 5-strand tripled semitendinosus and doubled gracilis autograft (5-STG), and 6-strand doubled gracilis and semitendinosus autograft plus allograft (6-STGAllo). The primary outcome measure was graft rupture.

RESULTS: 354 patients with an average age of 15.3 years (range 10 – 19 years) met inclusion criteria. There were 157 males and 198 females. Graft constructs included 4-STG (198 knees), 5-STG (91 knees), and 6-STGAllo (65 knees). Age differed across graft constructs with older patients more frequently represented among allograft-augmented grafts ($p<0.001$). The average diameter of the graft constructs was 8.3mm for 4-STG, 8.9mm for 5-STG, and 9.2mm 6-STGAllo ($p<0.001$).

Mean duration of follow-up was 26 months (range 6-56 months). 51 patients (14%) had ACL graft rupture. The graft failure rates were: 14% for 4-STG, 12% for 5-STG, and 20% for 6-STGAllo ($p = 0.51$). After adjusting for age and graft size, patients who had allograft augmented grafts (6-STGAllo) had 2.6 (95% CI: 1.02, 6.50) times the odds of graft rupture compared to 4-STG. There was no significant difference in failure rate between patients who had 5-STG grafts compared to 4-STG (OR=1.2; 95% CI: 0.5, 2.7).

CONCLUSION: ACL reconstructions with a hamstring autograft augmented with a soft tissue allograft have a significantly increased risk of graft rupture as compared to hamstring autograft without allograft augmentation. In situations where the surgeon harvests an inadequately sized 4-strand autograft, we recommend tripling the semitendinosus if length allows to produce a larger graft diameter rather than augmenting with an allograft.

89. Retroarticular Drilling With Supplemental Bone Marrow Aspirate Concentrate For The Treatment Of Osteochondritis Dissecans Of The Knee

Perkins, Crystal; Willimon, S. Clifton; Davidson, Kelsey; Grimm, Nathan; Christino, Melissa; and Busch, Michael

OBJECTIVES: A common treatment for immobile osteochondritis dissecans (OCD) lesions that have failed non-operative treatment is drilling. To our knowledge, supplementation of immobile OCD lesions with autogenous bone marrow aspirate concentrate (BMAC®) has not been described. The purpose of this study was to assess the radiographic healing of adolescent OCD lesions of the knee treated with retroarticular drilling and supplemental BMAC® to facilitate healing.

METHODS: Adolescent patients with OCD of the knee who underwent retroarticular drilling with supplemental BMAC® by two surgeons at a single institution were identified. Lesions were classified as small if < 320 mm² or large if >320 mm². All lesions were followed radiographically for evidence of healing. Healing was rated by two independent reviewers and based on the ROCK (Research in OsteoChondritis of the Knee) radiographic healing criteria.

RESULTS: 52 OCD lesions in 49 patients were included. 41 lesions were located on the medial femoral condyle and 11 on the lateral femoral condyle. Mean age was 12.5 (10 – 17) years. All lesions were categorized as immobile lesions based on the ROCK Arthroscopy Classification. The average lesion size was 407 (132 – 899) mm². The mean volume of BMAC® used per lesion was 6.4 (5 – 12) mL. 35 lesions (67%) were healed at a mean of 9.5 (1 – 47) months. 12 lesions (23%) were rated as 25-75% healed and 5 lesions (10%) at 0 – 25% healed at latest follow-up. 65% of large lesions and 72% of small lesions were healed at final follow-up. There were three minor complications (contact dermatitis, suture abscess and rash).

CONCLUSIONS: Healing rates following surgical treatment of OCD lesions are highly variable. Previously published series have shown that large lesions are significantly less likely to heal. Despite this we showed a 65% healing rate for large lesions in our series. We were not able to identify any predictive factors for failure of OCD lesions to heal. Retroarticular drilling of large OCD lesions of the knee with supplementation of BMAC® is a viable and safe alternative treatment which shows good results with no serious complications.

90. Maternal Poverty Effects on Developmental Epigenetic Age are Moderated by Neonatal Sex

Pilkay, Stefanie; Knight, Anna; Tylavsky, Frances; Bush, Nicole; and Smith, Alicia

BACKGROUND AND PURPOSE: Poverty in childhood predicts health and functioning in adulthood. Moreover, researchers have reported prenatal exposure to poverty predicts neonatal DNA methylation at birth. However, a new developmental epigenetic age offers a novel biological signature to investigate the effects of prenatal exposure to poverty on child development. Developmental epigenetic age uses DNA methylation to assess a “biological” age in reference to clinical estimates of gestational age. Moreover, a sex-specific timing of natural epigenetic processes including imprinting and masculinization of the brain calls for neonatal sex to be considered as a moderator of poverty effects on neonate developmental age. We sought to determine if prenatal exposure to poverty associates with developmental age in neonates and if neonate sex moderates the association.

METHODS: This research data is from the CANDLE Study, which was designed to examine early child-development risks. Maternal poverty was measured using household income in relation to the established national poverty line (above/below). DNA methylation was measured in umbilical cord blood in 216 neonates (55.0% African-American; mothers aged M=27, SD=5.15). Developmental age was as described by Knight et al. (2016). Linear regression assessed the effects of maternal poverty on neonates’ developmental age controlling for race and cell composition.

Results: Combined-sex analysis did not reach statistical significance for effects of prenatal exposure to poverty on developmental epigenetic age ($p>0.05$). However, sex-stratified analyses showed male neonates with a mother living below the poverty line have reduced developmental epigenetic age compared to males with a maternal SES above the poverty line ($B=-.99$, $SE=0.42$, $p=0.021$, bootstrap CI [-1.82, -.21]). Developmental epigenetic age did not associate with prenatal exposure to poverty in females ($p>0.05$).

CONCLUSIONS: The association of prenatal exposure to poverty effects and developmental age in neonates is moderated by sex. This may suggest males have increased susceptibility to the effects of prenatal poverty exposure.

91. Methods for Distinguishing Children’s Hospitals from Non-Children’s Hospitals

Piper, Kaitlin; Baxter, Katherine; McCarthy, Ian; and Raval, Mehul

BACKGROUND: Childrens hospitals (CH) provide high volume, specialized, and resource intense care to the sickest children. Though CH comprise less than 5% of all hospitals in the United States, they account for 40% of pediatric inpatient days and 50% of national pediatric healthcare costs. Because these hospitals represent a disproportionate amount of pediatric healthcare costs, it is important to determine if the high-cost, resource-intensive care provided by CH is justified by improved health outcomes. To compare health outcomes at CH and non-children’s hospitals

(NCH), the first methodological step is to classify hospitals into CH and NCH categories. However, the lack of a systematic and standardized process for classifying hospitals poses methodological challenges for these studies.

METHODS: Here, we describe a method for distinguishing CH from NCH. Using data from the 2015 American Hospital Association (AHA) Survey, 4,464 hospitals were classified into four categories (Tiers A-D). Tier A included hospitals that only provided care to children, Tier B included non-Tier A hospitals that had all key pediatric services (pediatric ED, PICU, and NICU), Tier C included non-Tier B hospitals that provided some key pediatric services, and Tier D hospitals provided no pediatric services. We then validated the classifications using publicly available hospital data as well as Health Care Cost Institute claims data.

Results: 51 hospitals were classified as Tier A, 228 as Tier B, 1,721 as Tier C, and 1,728 as Tier D. The majority of Tier A hospitals were members of the Children's Hospital Association (90.2%), while half of Tier B hospitals (52.6%) and very few Tier C/D hospitals were members (1.2% and 0.1%, respectively). Similar trends were seen for Children's Oncology Group and Pediatric Surgical Quality Improvement Program membership. Also, Tier A had the highest proportion of hospitals that provided pediatric organ transplants, provided congenital heart surgery, had verified pediatric trauma centers, and had NICU levels 3 or 4.

CONCLUSIONS: Using AHA survey data is a feasible and valid method for classifying hospitals into CH and NCH categories. Studies comparing health outcomes at CH and NCH can easily replicate this methodology for all years of the AHA survey.

92. Metabolic Dysfunction as a Contributor to 3q29 Deletion Syndrome Phenotypes

Pollak, Rebecca; Rutkowski, Timothy; Grewenow, Stephanie; Malone, Tamika; Purcell, Ryan; Pachura, Kimberly; Wynn, Grace; Caspary, Tamara; Dawson, Paul; Zwick, Michael; and Mulle, Jennifer

3q29 deletion syndrome (3q29DS) is a rare (~1:30,000) genomic disorder characterized by a 1.6 Mb heterozygous deletion on chromosome 3 and is associated with a wide range of phenotypes, from neurodevelopmental and neuropsychiatric disorders to reduced birth weight and growth deficits. Notably, the 3q29 deletion confers an ~40-fold increased risk for schizophrenia, a 30-fold increased risk for Autism Spectrum Disorder, and a significantly increased risk for intellectual disability and generalized anxiety disorder. To improve the current understanding of 3q29DS, the Emory 3q29 Project includes an online patient registry (3q29deletion.org), comprehensive in-person patient evaluations, and development of a novel mouse model of the syndrome. While the clinical presentation of 3q29DS is highly variable, the single unifying feature across affected individuals is growth deficits. We found that babies with 3q29DS weighed 14.9 ounces less at birth compared to babies in the general population ($p=5.55e-13$), and this reduced weight persists through childhood. This reduced birthweight and persistent low weight is robustly recapitulated in our mouse model of 3q29DS; mice with the orthologous 3q29 deletion weighed significantly less than wild type littermates at birth and through adulthood. The consistency of this phenotype, coupled with the fact

that there are three mitochondria-associated genes within the 3q29 interval (Tfrc, Bdh1, and Senp5), led us to hypothesize that there may be an unidentified metabolic disorder in individuals with 3q29DS. To answer this question, we have several ongoing studies of metabolic function in our mouse model, with future follow-up studies planned for human patients with 3q29DS. In addition to improving our understanding of 3q29DS-associated growth delay, gaining a better understanding of the metabolic phenotypes associated with the 3q29 deletion could potentially provide inroads to understanding more complex 3q29DS-associated phenotypes, specifically neuropsychiatric and neurodevelopmental disorders. While the relationship between metabolic and neuropsychiatric phenotypes is currently unknown, the strikingly increased neuropsychiatric risks and low birthweight associated with the 3q29 deletion suggest the possibility of a common causal linkage. Finally, as metabolic phenotypes can be amenable to therapeutic interventions, improving our knowledge of this core phenotype of 3q29DS may help us identify possible therapeutic targets for further study.

93. The Role of Bone Marrow Derived GAS6 in Resistance to MERTK Inhibition in Acute Myeloid Leukemia

Qiu, Annie; Minson, Katherine A; Huey, Madeline G; DeRyckere, Deborah; and Graham, Douglas K

Current research in treatment for acute myeloid leukemia (AML) focuses on novel targets, such as MERTK, a member of TAM family receptor tyrosine kinases (RTKs). Pharmacological inhibition of MERTK by a small molecule tyrosine kinase inhibitor, MRX-2843, prolongs survival in mice models, yet is not curative. The bone marrow microenvironment (BMM) plays a role in conferring therapeutic resistance and research implicates GAS6, a vitamin K dependent ligand of MERTK, as a mediator of this resistance. We hypothesize that bone marrow derived GAS6 protects leukemia cells in the BMM from therapeutic effects of MERTK inhibition and that inhibition of GAS6 will reverse this protection.

To determine the role of the BMM in protecting leukemia from anti-tumor effects of MRX-2843, the Kasumi-1 AML cell line was cultured in the presence or absence of a stromal cell line, HS27, with MRX-2843 or vehicle and analyzed by flow cytometry for apoptotic and dead cells. Co-culture with the stromal cell line protected AML cells from the MRX-2843 induced apoptosis seen in the absence of co-culture. Hs27 cells expressed low levels of GAS6 at baseline, which was increased when co-cultured with Kasumi-1. To evaluate the functional effect of GAS6 upregulation, Kasumi-1 cells were cultured with MRX-2843 and Hs27 conditioned medium or co-conditioned medium (from co-cultured Kasumi-1 + Hs27). Protection from MRX-2843 induced apoptosis did not differ in conditioned or co-conditioned cultures, suggesting that increased GAS6 expression does not have a functional role in mediating protection. Additionally, conditioned medium alone did not offer protection from apoptosis, supporting the notion that direct cell-cell contact between leukemia and stromal cells is necessary for this effect. The vitamin K antagonist, warfarin, was used as a translational inhibitor of GAS6. To measure the effect of GAS6 inhibition, Kasumi-1 and Hs27 cells were co-cultured in the presence of both MRX-2843 and warfarin. Warfarin-mediated GAS6 inhibition did not reverse protection against MRX-2843 induced apoptosis.

These data suggest that GAS6 is not a critical mediator of protection from MRX-2843 induced apoptosis. Future investigation into other ligands of MERTK, Protein S (PROS) and Galectin-3 (GAL-3) may offer insights into the mechanism of protection.

94. Cardiac Toxicity from Ethanol Exposure in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Rampoldi, Antonio; Singh, Monalisa; Wu, Qingling; Duan, Meixue; Jha, Rajneesh; Maxwell, Joshua; Zhang, Xiaoyu; Gibson, Greg; Brown, Lou Ann; and Xu, Chunhui

Alcohol use prior to and during pregnancy remains a significant societal problem which could lead to developmental fetal abnormalities including compromised myocardia function and increased risk for heart disease later in life. Alcohol-induced cardiac toxicity has traditionally been studied in animal-based models. These models have limitations due to physiological differences from human cardiomyocytes and are also not suitable for high throughput screening. We hypothesized that human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can serve as a useful tool to study alcohol-induced cardiac defects. In this study, hiPSC-CMs were treated with doses of ethanol, corresponding to the clinically relevant levels of alcohol intoxication. hiPSC-CMs exposed to ethanol showed a dose-dependent increase in cellular damage and decrease in cell viability, corresponding to increased oxidative stress and production of reactive oxygen species (ROS). Furthermore RNA-seq analysis showed significant alteration in genes belonging to the potassium voltage-gated channel family or solute carrier family, partially explaining an observed dose-dependent increase in irregular Ca²⁺ transients in ethanol treated hiPSC-CM. RNA-seq showed also significant up-regulation in the expression of genes associated with collagen and extracellular matrix (ECM) modeling (MMP9, EMID1 and COL14A1), and downregulation of genes involved in cardiovascular system development (NPPB, DNAAF3) and actin filament-based process (NPPB, DNAAF3). These results suggest that hiPSC-CMs could be a novel, physiologically relevant system for the study of alcohol-induced cardiac toxicity and have the potential to be used as a platform in high-throughput screening for disease modeling and drug discovery.

95. Elevated Arginase Activity and Left Ventricular Hypertrophy in Children with Chronic Kidney Disease

Reyes, Loretta; Winterberg, Pamela; Brown, Lou Ann; Harris, Frank; Kelleman, Michael; and Morris, Claudia

INTRODUCTION: Left ventricular hypertrophy (LVH) is common in pediatric chronic kidney disease (CKD) and end stage renal disease (ESRD) patients and is an independent risk factor for cardiovascular morbidity and mortality. Nitric oxide (NO), a vasoactive substance critical for vascular homeostasis, is synthesized from arginine by NO synthase enzymes. Arginine can also be

catabolized by arginase enzymes, thereby reducing NO bioavailability. Since the kidneys play a key role in endogenous arginine synthesis, we hypothesized that arginine bioavailability is altered during CKD/ESRD and predicts cardiovascular complications.

METHODS: Banked plasma from children with (n=47) and without (n=11) CKD/ESRD was analyzed for metabolites of arginine biosynthesis by mass spectrometry; arginase concentration and activity were measured by ELISA and colorimetric assay respectively. Data was correlated with measures of LVH from echocardiograms in CKD/ESRD patients.

RESULTS: Arginase activity was significantly higher in CKD/ESRD patients compared to healthy children (3.12 units/L vs 1.52 units/L, p=0.008). Dialysis patients had a lower arginase concentration compared to normal controls, but arginase activity was significantly increased (p=0.04). Arginase activity was increased in patients with LVH (p=0.07) and had a trending association with left ventricular mass index (LVMI) z-score on Spearman correlation [r= 0.339; p=0.06; n=31].

CONCLUSION: In this pilot, we found that arginase activity was significantly increased in children with CKD and ESRD and may be associated with LVH, an observation that has not been previously reported. Interestingly, arginase activity was disproportionately higher than its concentration in ESRD patients on dialysis compared to pre-dialysis CKD patients, suggesting a mechanistic activation of arginase enzyme activity in dialysis patients.

Translational Impact: Increased arginase activity during CKD and ESRD may divert arginine away from NO synthesis. Therapeutic inhibition of arginase activity may ameliorate cardiovascular complications in children with chronic kidney disease.

96. Mechanisms of Cell Death Induced by Engineered Reovirus in Triple-Negative Breast Cancer
Rodriguez Stewart, Roxana M.; Berger, Angela K.; Guberman, Jaime; and Mainou, Bernardo A.

Triple-negative breast cancer constitutes approximately 15% of all breast cancer and is associated with worse prognosis when compared to other subtypes of breast cancer. There is a need for targeted therapeutics to treat this type of breast cancer, as current therapies are largely limited to cytotoxic chemotherapy. Mammalian orthoreovirus (reovirus), a nonenveloped segmented dsRNA virus in the Reoviridae family causes a mostly asymptomatic infection in humans. Reovirus has been shown to preferentially kill transformed cells and is currently in Phase I-III clinical trials to assess its efficacy as an oncolytic against a variety of cancers. To engineer reovirus with enhanced infective and cytopathic properties against triple-negative breast cancer cells, we coinfecting triple-negative breast cancer MDA-MB-231 cells with parental reoviruses T1L, T2J, and T3D. Following sequential serial passage, we isolated reassortant reovirus r2Reovirus. r2Reovirus has genomic segments predominantly from T1L with one gene segment from T3D and synonymous and nonsynonymous point mutations. Infection of MDA-MB-231 cells with r2Reovirus is more efficient and induces greater cell death than parental reoviruses. Infection by r2Reovirus induces caspase-dependent apoptotic cell death in MDA-MB-231 cells. Our data show that a genetically engineered reovirus can

more efficiently induce programmed cell death in a subtype of triple-negative breast cancer. In the long term, we aim to identify the contribution of specific host and viral factors in reovirus-mediated cell death of triple-negative breast cancer cells.

97. What do Patients Want to Know about Sickle Cell Disease and Desired Methods of Learning, a National Study

Ross, Diana; Bakshi, Nitya; Sinha, Cynthia; Khemani, Kirshma; and Krishnamurti, Lakshmanan

BACKGROUND: Monitoring and management of chronic complications of sickle cell disease (SCD) can be complex, involving a multi-disciplinary approach along with shared patient decision making. Understanding by the patient and caregiver of SCD is the corner-stone of quality decision-making.

PURPOSE: 1. To evaluate information patients and caregivers find relevant to sickle cell care and management. 2. To understand the methods for learning preferred by patients and caregivers

METHODS: We conducted qualitative interviews with geographically dispersed participants recruited from national conferences and clinical practice. Interviews were audio recorded and transcribed verbatim. NVivo10.0 was used to manage data for analysis. Thematic analysis was used to analyze 179 patients and caregivers interviews, age 18 to 67 years, 68% female).

RESULTS: Several themes related to patient and caregiver learning were identified. 1. Physician-provided education is good but is limited by the duration of the clinical appointment and does not address all the informational needs of patients and caregivers.. 2. Participants want to learn at their own pace, and in-depth. They use the internet extensively for information but are uncertain of the credibility of available information. 3. Unbiased Video Testimonial from patients and caregivers preferred and considered more credible than health care provider perspective. 4. Strong preference for a web based resource that provides high quality unbiased information presented in an easily understood, visually feasible, format which is easy to use. 5. Preference for web-based tool to provide information about SCD, complications, self-management, treatment options, including risks, benefits, expected outcomes, and long-term side effects. 6. Web-based tool should also help users clarify their values and guide them through the steps of the decision-making process.

CONCLUSIONS: Patients with SCD and their caregivers expressed the need for an educational resource that will supplement education by healthcare providers. They would like the resource to be web-based, credible, accurate, easy to use and enriched for providing the perspective of patients and caregivers perspective on dealing with therapeutic decisions.

98. What do Patients Consider Important in Making Medical Decisions about Sickle Cell Disease: Results of a Nationwide Study?

Ross, Diana; Bakshi, Nitya; Khemani, Kirshma; and Krishnamurti, Lakshmanan

BACKGROUND: Shared medical decision making by physicians and patients requires quality information about disease process and treatment options. It takes into account what patients consider important. It also minimizes decisional conflict, and decisional regret. The purpose of this study was to identify and begin to understand what patient values are in treatment decision-making.

METHODS: We conducted qualitative interviews with geographically dispersed participants recruited from national conferences and seminars to inform development of a decision aid tool. Interviews were audio recorded and transcribed verbatim. NVivo10.0 was used to manage data for analysis. We analyzed 60 transcripts; 6 stakeholders, 22 caregivers, 32 patients; 72% female.

RESULTS: Eight themes were identified: 1) Current health status and life-threatening complications lead patients to seek treatment. Potential benefits are weighed against risk of severe complications, new illnesses, and death. 2) Physical appearance can prompt patients to seek treatment while side effects may discourage treatment selection. 3) Fertility preservation when considering hydroxyurea or bone marrow transplantation. Parents of children identified a burden of concern when weighing current care needs against the potential infertility of their child. 4) Burden of care as well as missed school and work negatively influence treatment choice. There is an uneasy comfort with the status quo related to burden of care in terms of unpredictable events. Expressed concerns regarding burden of care were related to predetermined interruptions that correlate with treatment options, such as monthly clinic appointments and blood draws. 5) Disruption of family routines and activities have a negative impact on family function and were reasons for considering treatment options. 6) Limited ability to participate in social activities was described as an impairment to quality of life causing consideration of treatment changes. 7) Feelings of depression and isolation caused by disease complications and burden did not seem to be enough to always make a treatment choice. 8) Impact on family financial health due to out-of-pocket expenses impede treatment choice.

CONCLUSIONS: We have elicited several factors that families consider important while making medical decisions. Paying attention to and addressing these issues may be beneficial in enabling the making of shared medical decisions.

99. Patient and Caregiver Understanding of Shared Decision Making for Sickle Cell Disease: A Qualitative Study

Ross, Diana; Bakshi, Nitya; Sinha, Cynthia; and Krishnamurti, Lakshmanan

BACKGROUND: Shared decision-making (SDM) is a collaborative approach between patients and healthcare providers (HCPS) to determine patient treatment. There are three basic elements of communication for SDM: 1) A decision is needed, 2) Risks, benefits, and efficacy are understood, 3) Provider recommendations and patient values and preferences are considered. The objective of this study was to understand patient interpretation of SDM.

METHODS: We analyzed qualitative interviews of patients/ caregivers from a geographically diverse population recruited from national conferences and local clinics. A semi-structured interview guide was used to collect data. Audio recordings were transcribed verbatim. Transcripts were coded using content analysis with NVivo10.

RESULTS: We enrolled 49 participants, 48 were African American, median age 37 (range 19 to 66), 41 females. Forty-three had at least some college, 10 had bachelors, and 16 were post-graduates. Most participants never heard of SDM nor did they explicitly understand its meaning. For those who have heard of it, SDM represents shared responsibility between the patient/parent and the physician to make an informed decision about healthcare needs. All participants indicated a strong desire to be involved in SDM and many noted that patients have the final say. The role of physicians is to introduce treatments options or test procedures, including risks and benefits. The role of patients/parents is to discuss and understand risks and benefits. Most identified a need to do their own research, which included talking with other patients. Most participants learned to make medical decisions out of necessity as they became adults or had an infant with sickle cell disease (SCD). Most expressed themselves as being fairly confident they make good decisions stating validation through outcomes such as improved blood counts and no side effects. They also cited their health as a factor in confidence, stating this was of the utmost importance.

CONCLUSIONS: Patients with SCD and their parents in this study expressed a desire to be part of the SDM process. They believed that the physician's role is to give information about treatment options and risks and benefits and it is for the patient to decide.

100. Optical Pacing of Cardiomyocytes Using mRNA

Sayegh, Michael; Beyersdorf, Jared; Wolfson, David; Fernandez, Natasha; Santangelo, Philip; Cho, Hee Cheol; and Santangelo, Philip.

Optogenetics offers a non-invasive approach to control the electrical excitation and mechanical contraction of cardiac cells in vitro and in vivo. In this work, we employ in vitro transcribed mRNA to transiently express opsins in neonatal rat ventricular myocytes-an in vitro model of cardiac tissue. We show that Viromer Red preferentially transfects cardiomyocytes compared to fibroblasts, whereas no cell-specific difference is seen with lipofectamine (L2K), another commonly used lipid transfection reagent. We visualize the translation and membrane localization of the ultra light-sensitive and rapidly activated calcium-permeable channelrhodopsin (CatCh), delivered via Viromer Red mRNA transfection. Using longitudinal flow cytometry, we find that mRNA delivered CatCh is maximally expressed at the end of a 24 hr pulse of mRNA in >80% of cardiomyocytes compared to <30% of fibroblasts. Furthermore, the expression per cell, as measured by mean fluorescence intensity (MFI) of cells stained for CatCh, was >2 times higher in cardiomyocytes compared to fibroblasts at 24 hrs. Using multi-electrode arrays (MEA) to record extracellular potentials in response to light stimulation, we were able to pace and drive NRVM monolayers at a 100% capture fidelity starting at 12 hrs post transfection with a rectangular light pulse train of 2 Hz frequency. We found a negative correlation between the threshold light intensity and light pulse duration required

to pace and drive NRVM monolayers. This strength-duration relationship exhibited exponential-like shape typical of electrically excitable membranes. Compared to an older opsin variant, channelrhodopsin 2 (ChR2), CatCh required lower light intensity and shorter light pulse durations for successful pacing for up to seven days post-transfection. Out of three doses of CatCh in vitro (0.1, 1 and 10 ng/1k cells), we determined 1 ng/1k cells to be the optimal dose based on light intensity thresholds, percent of samples captured and persistence of light excitability over time. Thus, we posit that we can successfully pace cardiac cells in vitro with light using an mRNA encoded opsin.

101. Interpretable Predictions for Cardiac Arrest Using Deep Convolutional Networks

Sha, Ying; Chen, Johnny; Tong, Li; and Wang, May

Cardiac arrest is a common heart disease complication with an extremely low overall survival rate, because it occurs so abruptly so that only limited time is left for healthcare providers or even bystanders to react. The need for early detection or assessment of future cardiac arrest is very strong. Though much effort has been devoted to improve survival after in-hospital cardiac arrest, the survival-to-hospital-discharge rate is still unsatisfactory (e.g., the risk-adjusted rates of survival to discharge increase from 14.3% in 2000 to 43.4% in 2009, but it is less than 50%). Recently, Children's Healthcare of Atlanta (CHOA) has deployed the BedMaster system manufactured by Excel Medical Electronics, which acquires and stores high frequency vital signs waveform for continuous bedside monitoring of patients in the Cardiac Intensive Care Unit (CICU). The deployment of BedMaster provides opportunities for using advanced data analytics to capture fine variations in patients' conditions.

In this project, we propose to apply deep convolutional neural network (CNN) on high-frequency vital sign data. Deep CNN can learn feature representations without manual feature selection or engineering. However, the successful adoption of analytics in biomedical research community requires explainable predictions that is usually hindered by the black-box properties of deep neural networks. Therefore, we use a post-hoc interpretation methods named Grad-CAM, which multiples the gradients flowing to the last convolutional layer with the weight activation of feature maps in the last convolutional layer to highlight the regions impactful for individual predictions. We have implemented and evaluated this analysis framework on PhysioNet 2017 Challenge data set, which contains high-frequency ECG, to generate preliminary results. We got a F1 score of 0.8 and visualize predictions. We plan to deploy this analysis framework on BedMaster data from CHOA for early detection of cardiac arrest.

102. Harnessing an Innate Frog Defense Peptide as an Anti-Influenza Agent

Shartouny, Jessica; Lee, Song-Hee; Holthausen, David; and Jacob, Joshy

Influenza infections are responsible for much of the annual disease and economic burden worldwide. In the 2017-2018 flu season, over one hundred children in the United States died and children aged 0-4 were hospitalized at a higher rate with influenza-like-illness than other

demographics, often developing complications. The first defense against influenza is vaccination. Children as young as 6 months old can be vaccinated, though they may require two doses. However in pandemics, when the predicted vaccine strains are mismatched, or if the child has not been vaccinated or did not respond well, antiviral drugs become the next option. Resistance to conventional antiviral agents can develop in a number of influenza subtypes, necessitating the development of novel anti-viral compounds. One source of new agents is in the innate immune repertoire of antimicrobial peptides (AMPs) produced by most forms of life that are involved in the initial response to bacterial pathogens. We have developed a library of AMPs derived from skin secretions of Indian frogs, from which we previously reported an anti-H1N1 peptide. Another peptide, Yodha, has demonstrated potent neutralization activity against H3N2 influenza viruses in in vitro assays. Yodha also neutralizes H1N1 and two flaviviruses, indicating that its mechanism is broadly active. In TEM images, we see that Peptide 47 appears to form nets around viruses, which may inhibit cellular entry. As Peptide 47 is also nontoxic to human red blood cells to a high concentration, it is a good candidate for antiviral therapeutic development.

103. Research @ the Medical Robotics and Automation (RoboMed) Laboratory

Sheng, Jun; Cheng, Shing Shin; Park, Kihan; Chitalia, Yash; Wang, Xuefeng; and Desai, Jaydev

The Medical Robotics and Automation (RoboMed) Laboratory has been actively working in the development of automated medical diagnostic tools and meso-scale robots for minimally invasive surgery (MIS), with potential applications in pediatrics. A portable diagnostic device that combines MEMS technology with tissue characterization was developed for breast cancer diagnosis. Since the device can capture the changes in the mechanical, electrical and thermal properties of diseased tissues, it can potentially be used to diagnose a disease by phenotyping a tissue sample from the pediatric biopsy. The minimally invasive neurosurgical intracranial robot (MINIR-II) is a flexible surgical robot designed to remove deep-seated brain tumor. Completely 3-D printed and MRI-compatible, it is equipped with electrocautery probes and suction tubes, and envisioned to be guided under MRI. A novel transmission mechanism has been developed to remotely operate the MINIR-II to ensure artifact-free MR images. Another robot for intracranial neurosurgery was developed for neurosurgical intracerebral hemorrhage evacuation (NICHE). The robot end effector can articulate within the hemorrhage through an SMA torsion joint and an SMA bending joint. A headframe interfaces with the NICHE robot, allowing precise targeting of the hemorrhage. To realize feedback control of the torsion joint, a fiberoptic rotation sensor based on light intensity modulation has been developed. For cardiovascular MIS, a robotic guidewire was developed for the treatment of peripheral artery disease (PAD). PAD is caused due to the blocking of the arteries in the lower extremities of a human body. It requires the operating clinician to manually navigate a thin flexible wire called the 'guidewire' through long tortuous vasculature. The tip of the developed guidewire is 0.78 mm in diameter and has two degrees of freedom. By pulling and releasing tendons, the bending joints of the guidewire tip can be remotely controlled. In addition, a robotic cardiac catheter was developed for atrial fibrillation diagnosis and treatment. Its steerable tip is formed by multiple bending modules and each module is actuated by SMA wires. By heating SMA wires using

Nichrome coils through conductive heating, low heating current is required and MRI compatibility is improved.

104. Targeting the YAP/Hippo Pathway to Enhance Precision Therapeutics in Neuroblastoma

Shim, Jenny; Lee, Yoo Joo; Fritz, Alexa; and Goldsmith, Kelly C.

Relapsed high-risk neuroblastoma (rNB) portends a poor outcome, underscoring the critical need for novel therapeutics. Genetic alterations in RAS-RAF-MAPK are significantly higher at relapse; yet, targeted therapy with MEK inhibitors alone has proven less than effective clinically. rNBs also have increased Yes-associated Protein (YAP) transcriptional activity. YAP is a transcriptional co-activator that binds with TEA Domain (TEAD) family transcription factors in the nucleus to regulate genes promoting tumor initiation, cell proliferation, and survival. YAP can also promote resistance to MEK-targeting agents in adult cancers. We sought to characterize YAP's role in tumor initiation, cell proliferation, and therapy resistance in rNB.

Methods: Baseline YAP-expressing and YAP-null human neuroblastoma cells were cultured in neural basal media to form neurospheres or in RPMI media. YAP transcriptional levels were quantified. NF-1 and NRAS-mutated neuroblastoma cells were used to create stable YAP knockdown and YAP-expression models via lentiviral transduction. These cell models were evaluated for changes in proliferation, tumor initiation, and response to Trametinib (MEK inhibitor) and standard chemotherapy.

RESULTS: Unmodified neuroblastoma cells grown as a neurosphere developed increased YAP expression compared to the same neuroblastoma cells grown as a monolayer in culture. While cell proliferation in vitro was not affected, shRNA inhibition of YAP significantly decreased time to tumor formation in vivo compared to xenografts of empty vector-transduced cells. YAP knockdown sensitized RAS and NF-1-mutated neuroblastomas to Trametinib and chemotherapy while YAP over-expression induced therapy resistance in vitro. These data support the tumor initiating and highly therapy resistant properties instilled by YAP in rNBs.

CONCLUSIONS: The resistance of RAS/MAPK mutated rNBs to single agent MEK inhibitors supports the need for combination therapies to target the solid tumor heterogeneity, despite having an actionable mutation. Given the relevance of YAP in rNB and its role in therapy resistance, the need for YAP inhibition in combination with precision therapies like Trametinib is crucial. Given the lack of bona fide YAP inhibitors to date, we are currently chemically optimizing a YAP polypeptidomimetic to have enhanced permeability, nuclear localization, and TEAD affinity to create a bonafide YAP inhibitor for such combination preclinical testing.

105. System Biology and Computational Approaches to Predicting Reparative Potential of Stem Cells

Shoja-Taheri, Farnaz; George, Alex; Platt, Manu; and Davis, Michael

Congenital heart defects (CHD) are among the most common reasons of mortality in newborns and despite advanced surgical treatments, many patients experience heart failure. Currently, transplantation is the most effective cure for heart defects which has its own limitations such as the possibility of organ rejection. Stem cell/progenitor cell therapy is a novel method in treating heart failure in congenital heart patients. However, choosing stem cells with highest regenerative effects has been a challenge and there is tremendous patient-to-patient variability. We previously showed the age-dependent regenerative effects of human cardiac progenitor cells (hCPCs) in a rat model of juvenile heart failure. In the same study, using a small subset of patients, computational modeling analysis showed that regression models could be made linking sequencing data to phenotypic outcomes. In the current study, we used that quantitative model to determine whether predictions can be made in a larger population of patients regarding cell function in neonatal hCPCs. The goal of this study was to validate the functional responses in each neonatal hCPC line and confirm our model. We performed RNAseq from CPCs isolated from 8 different neonatal patients. We tested 2 functional parameters of our model, proliferation and chemotactic potential of conditioned media using Click-it Edu and Boyden chamber assays, respectively. Interestingly, the observed proliferation and migration responses in each of the selected neonatal hCPC lines matched their predicted counterparts. Furthermore, in vitro analysis verified our model in that one of the neonate hCPCs performed far worse than other patients as predicted. Altogether, these data show that cell behavior may be predicted using large data like RNAseq, and that we may be able to identify patients that may exceed or underperform expectations. With systems biology approaches, interventions can be tailored to improve cell therapy, or mimic the qualities of reparative cells.

106. DNA Methylation Signatures of Pediatric Crohn's Disease Largely a Consequence of Inflammation

Somineni, Hari K.; Venkateswaran, Suresh; Kilaru, Varun; Marigorta, Urko M.; Mondal, Kajari; Okou, David T.; Hams, Jeffrey S.; Denson, Lee A.; Cutler, David J.; Gibson, Greg; Conneely, Karen N.; Smith, Alicia K.; and Kugathasan, Subra

Somineni, Hari; Ven

BACKGROUND: Crohn's disease (CD) is a life-long relapsing-remitting disorder with flares of inflammation and a heterogeneous clinical course. A subgroup of patients with CD progress from an inflammatory phenotype (B1) to stricturing (B2) behavior. DNA methylation differences have been reported in CD but their role remains unelucidated; thus, it is critical to investigate the temporal relationship between methylation and disease to establish whether the methylome plays a causal role and can be leveraged for therapeutic benefits.

METHODS: Utilizing a subset of subjects recruited in the pediatric RISK study we generated genome-wide DNA methylation data using the Illumina HumanMethylationEPIC 850K array in blood DNA samples of 74 controls and 164 newly diagnosed CD cases (B1), of which 55 progressed to B2 within 36 months from diagnosis. We used genetic association and the concept of Mendelian randomization to test for causal relationships between methylation changes and CD.

RESULTS: We found differential methylation at 1,189 CpGs associated with CD at diagnosis (FDR<0.05). Estimated effects of methylation changes at these CpGs showed a strong correlation with plasma C-reactive protein levels, a marker of inflammation (R=0.91; P<2.2x10⁻¹⁶). When we performed within cases analysis comparing their methylation profiles at diagnosis to follow-up, the sign of the effect has reversed, while the magnitude of the change has remained the same at nearly all 1,189 sites (R=-0.93, P<2.2x10⁻¹⁶). We noted similar results even after stratifying patients based on disease progression to B2 (R=-0.91, P<2.2x10⁻¹⁶ for progressors; R=-0.90, P<2.2x10⁻¹⁶ for non-progressors). We further demonstrated that, while some methylation changes associated with CD might be causal, the vast majority are the consequence of inflammation.

CONCLUSIONS: We show convincing evidence that the signatures of inflammation appear to primarily be a symptom of the disease rather than a cause, suggesting that treatment of inflammation may fundamentally be treating the symptoms of CD rather than the cause. This may at least partially explain why CD often remains a life-long remitting and relapsing disorder, despite effective treatment of the inflammation symptoms.

107. Mapping a Path to Precision in Childhood Cancer: The Aflac Cancer and Blood Disorders Center Precision Medicine Program

Summers, Ryan J.; Felker, James; Mitchell, Sarah; Park, Sunita; Carter, Alexis; Goldsmith, Kelly C.; MacDonald, Tobey; Pauly, Melinda; Cash, Thomas; Aguilera, Dolly; Porter, Christopher C.; Castellino, Sharon M.; Wechsler, Daniel S.; and Graham, Douglas K

Knowledge in tumorigenesis is rapidly evolving with novel diagnostics and discoveries in de novo disease, cancer progression, and relapse. However, clinical translation into targeted treatment of these tumors has lagged in pediatric cancer, in part due to the high complexity of the results of new tumor profiling platforms. To address these challenges we formed the Aflac Precision Medicine Program (APMP). The APMP uses comprehensive, integrated tumor profiling coupled with a multidisciplinary team in a Molecular Tumor Board (MTB) to identify molecular targets with the aim of improving the outcomes of children with de novo or recurrent high-risk tumors. The MTB facilitates discussion of tumor profiling data to provide evidence-based treatment recommendations to the treating oncologist. A selection of cases has highlighted challenges facing clinicians in the era of tumor genomic data.

Case 1: A patient with metastatic carcinoid tumor had molecular profiling performed at three different laboratories, twice from diagnostic tissue and once from relapse. An alteration in KRAS of unclear oncogenic potential was identified by all three platforms. An additional alteration in ARID1A was identified in a metastatic lesion at relapse. This case underscores the challenges associated with lesions of unclear functional significance and for which targeted agents are not available.

Case 2: A patient with relapsed mixed-phenotype acute leukemia underwent genetic testing which revealed a variant in TP53 that has been reported in several Li-Fraumeni families. This raises

concern for a germline mutation in TP53 and has implications for the patient's future treatment and the family if confirmed.

Case 3: A patient with a low-grade glioma had clinical progression to a high-grade astrocytoma. While somatic tumor testing at initial diagnosis revealed a BRAF V600E mutation, the patient did not respond to targeted therapy. Repeat tumor genetic testing at progression failed to identify a BRAF mutation but revealed a novel NTRK2 fusion, which was targeted with a dramatic clinical response.

These cases illustrate the potential challenges and benefits associated with genomic tumor profiling. The establishment of the APMP and the multidisciplinary expertise of an MTB is expected to enhance the care of children with high-risk tumors.

108. Combating Liver-Stage Malaria with Compounds Isolated from a Cyanobacterium *Lyngbya* sp.

Sweeney-Jones, Anne Marie; Gagaring, Kerstin; McNamara, Case; and Kubanek, Julia

According to the 2017 World Malaria Report released by the World Health Organization, an estimated 216 million cases of malaria occurred in 2016 which resulted in about 445,000 deaths. Children under the age of five are especially susceptible to infection and account for a large portion of the total reported deaths. Drug resistance and the limited treatment options for the asymptomatic liver stage of infection are a persistent problem facing efforts to eradicate malaria. Two species of the malaria-causing protozoa *Plasmodium*, *P. vivax* and *P. ovale*, can form hypnozoites, a dormant form of the parasite's sporozoite life stage located in the host's liver. Hypnozoites do not cause symptoms, cannot be detected by blood tests, and can trigger a new occurrence of malaria months after the initial infection. Novel antimalarial drugs are needed to target the liver stage of the parasite life cycle to protect populations in malaria endemic regions.

Organisms have proven to be an invaluable source for compounds with antimalarial bioactivity. For example, one of the standard pharmaceuticals used for treatment of the species *P. falciparum*, the endoperoxide sesquiterpene artemisinin, comes from the terrestrial plant *Artemisia annua*. A promising source for new antimalarial compounds are marine organisms, which produce a wide variety of secondary metabolites with unique features that are specific to the marine environment. In fact, our lab has previously isolated antimalarial bromophycolides from the red alga *Callophycus serratus*.

Screening a total of 1550 marine macroorganism extracts led to the identification of 124 extracts with activity against sporozoites of *P. berghei*, a species often used as a model organism for human malaria infection. The most promising compounds have come from a cyanobacterium *Lyngbya* sp. collected from the Lau Islands of Fiji which not only exhibited high potency towards *P. berghei* sporozoites but also had low toxicity towards human liver cells. Fractionation of the antimalarial *Lyngbya* sp. extracts led to the isolation of two bioactive peptides, a cyclic depsipeptide containing

phenylalanine, valine, and thiazole and a modified linear peptide containing leucine, isoleucine, and O-methyl tyrosine. Efforts are currently ongoing to fully characterize the bioactive compounds.

109. Visual-Spatial Memory and Child Pedestrian Injury Risk

Tang, Kerri; and Schwebel, David C.

BACKGROUND: In the United States, 223 children died from pedestrian injuries in 2015, equivalent to 21% of total child deaths from traffic crashes (NHTSA, 2017). The causes of child pedestrian injury are varied, but one reason children are at greater risk for pedestrian injury compared to adults is because their cognitive-perceptual-motor skills are underdeveloped. Much of the focus on child pedestrian research has been placed on improving these cognitive skills, but the field still has a poor understanding of which skills are most critical for training purposes. In particular, little is known about the role of visual-spatial memory in child pedestrian injury risk.

METHODS: As part of a larger study on child pedestrian safety, 117 children ages 7-8 completed “Mr. Peanut”, an interactive computer-based game that assesses children’s visual-spatial memory (Doherty-Sneddon & Bonner, 2001). In the game, a figure (Mr. Peanut) is shown with several colored stickers on different parts of his body. The stickers are then removed and Mr. Peanut appears “blank”, without stickers. Children are asked to recall the position of the colored stickers that were previously shown.

Children also crossed a simulated virtual reality pedestrian environment with two-lane bidirectional traffic 24 times (Schwebel et al., 2016). The percentage of crossings with hits/close calls (times when the child was <1 second of being struck by simulated vehicle) was recorded.

RESULTS: Children’s visual-spatial memory, as assessed on the Mr. Peanut task, was strongly negatively correlated ($r(115)=0.34$, $p<0.01$) with pedestrian injury risk. Sensitivity partial correlation analyses with age partialled, with gender partialled, and with age and gender partialled together all yielded similar results ($r=0.34$, $p<0.01$ in all analyses).

CONCLUSIONS: Visual-spatial memory is associated with child pedestrian injury risk: Children with poorer visual-spatial memory made more errors in crossing the street, even after controlling for age. This finding is logical, as safe pedestrian engagement requires memory of the traffic situation on one side (e.g., left) while viewing and processing visual-spatial information on the other (e.g., right). Efforts to teach children visual-spatial memory skills, either through pedestrian-related simulation/virtual reality or more traditional cognitive training strategies, could improve child pedestrian safety.

110. Automatic Diagnosis of Heart Rejection using Whole-Slide Images with Multi-Scale Context-Aware Features

Tong, Li; Sha, Ying; Deshpande, Shriprasad; and Wang, May

Heart transplantation is considered as the only cure for end-stage heart failure. In pediatric patients, approximately 400 heart transplants are performed every year in North America. The most common cause of mortality and morbidity in the pediatric heart transplant population is a rejection of the donor's heart by the recipient. Early and accurate identification of rejection is critical to preserving the transplanted heart, guiding therapy and ultimately, saving the life of the patient. The gold standard for diagnosing heart transplant rejection, established by the International Society of Heart and Lung Transplant (ISHLT), is a direct pathologic analysis of endomyocardial biopsy samples (EMB). The procedure of EMB collection and analysis is routinely performed in the cardiac catheterization laboratory. However, manual examination of the whole-slide images (WSIs) by pathologists can be time-consuming and prone to errors.

Advances in digital imaging analysis and machine learning have provided us with additional tools in evaluating various pathologies. In this project, we propose to apply convolutional neural network (CNN) with multi-scale context-aware features to the WSIs for automatic and accurate diagnosis of heart rejection. The image pyramid is one of the major characters of WSIs, which can be used to build feature pyramids. The feature pyramid is a basic component in recognition systems for detecting objects at different scales. For feature extraction in our heart rejection diagnosis task, we combine image patches of the WSI from different resolution levels. The image patches are tiled from the same region of interest (ROI). Thus, these image tiles have the same geographical center. The lower-resolution image patches have larger fields of view (FOV), which provide the neighborhood information for the high-resolution image patch. We then use CNNs to extract features from the image patches at different resolution levels and then combine the extracted features at either early stage of the convolutional layers or the late stage of the convolutional layers. Preliminary results suggest that the extra neighborhood information from the lower resolution images boosts the prediction accuracy of heart rejection.

111. Prospective Fontan Surgical Planning: Challenges and Accuracy

Trusty, Phillip; Slesnick, Timothy; Wei, Zhenglun; Rossignac, Jarek; Kanter, Kirk; Fogel, Mark; and Yoganathan, Ajit

The Fontan procedure is the current operation of choice for children born with single ventricle congenital heart defects. Surgical planning has been used over the past decade to provide additional insights into the clinical decision-making process. In its current state, surgical planning offers an accurate hemodynamic assessment of the preoperative condition, provides anatomical constraints for potential surgical options, and decent postoperative predictions if boundary conditions are similar enough between the preoperative and postoperative states.

The surgical planning process is a multi-step process that involves collaboration between clinicians and engineers. The major steps include preoperative image acquisition, image processing, creation of virtual surgical options, and numerical simulations of those proposed options. Current methodologies for anatomy prediction vary from simple computer programs to advanced solid

modeling software designed specifically for Fontan surgical planning, while blood flow prediction methods range from using preoperative waveforms to sophisticated lumped parameter modeling.

The planning process, data flow and time requirements are important considerations that must be understood to effectively implement surgical planning. For the simplest cases with high quality imaging data, the process requires approximately 60 hours, with 10 hours of user input. Naturally, as more surgical options are modeled at increasingly accurate physiological settings, this time will increase.

Thus far, surgical planning has been used on approximately 70 cases, with around 15 patients returning for follow up imaging. Surgical planning predictions were found to be more accurate for certain surgical options, and highly correlated with variations between the predicted and surgical implementation. Anatomy prediction was found to be a primary factor in prediction error.

Moving forward, validation with postoperative data is a necessary step in order to assess the accuracy of surgical planning and determine which methodological improvements are needed. Future efforts to automate the surgical planning process will reduce the individual expertise needed and encourage use in the clinic by clinicians. As postoperative physiologic predictions improve, Fontan surgical planning will become an even more effective tool to accurately model patient specific hemodynamics.

112. Mechanisms of Resistance to WEE1 Inhibition in Acute Leukemia Cells

Ulusik, Rizvan C.; van Linden, Annemie A.; Jones, Kenneth L.; Venkataraman, Sujatha; Vibhakar, Rajeev; and Porter, Christopher C.

WEE1 is a cell cycle kinase and DNA damage checkpoint kinase. Therapeutic strategies targeting the protein have revealed promising preclinical results. A small molecule AZD1775 is a WEE1 inhibitor tested on several different cancer types in combination with standard and targeted therapy. In anticipation of clinical application, this work investigates the mechanistic aspects of acquired resistance to WEE1 inhibition by AZD1775 in acute leukemia cells.

We generated 3 cell lines with acquired resistance to WEE1 inhibition by culturing cells in AZD1775 at increasing concentrations over the course of 4 months. We found that AZD1775 resistant cell lines are dependent upon increased HDAC activity, in part due to increased KDM5A activity. In addition, gene expression analyses demonstrate HDAC dependent increase in c-MYC expression and activity in AZD1775 treated resistant cells. Pharmacologic inhibition of BRD4, and thereby c-MYC, abrogated resistance to AZD1775. A synergistic effect of AXL inhibitor TP0903 with AZD1775 reported to abrogate the resistance of WEE1 inhibition in small-cell lung cancer. In contrast, in this study, drug treatments with TP0903 and AZD1775 showed an antagonistic effect on tested acute lymphoblastic resistant cell lines indicating the AXL pathway is not involved in acquired resistance in acute leukemia cells.

These studies suggest that HDAC inhibition can be combined with AZD1775 to restore sensitivity to the WEE1 inhibitor, if resistance develops.

113. Total Vascular Resistance in Single Ventricle Patients

Vaiyani, Danish; Matsuo, Kumiyo; Patel, Bhavesh; Akintoye, Olalade; Travers, Curtis; Sachdeva, Ritu; and Petit, Christopher

BACKGROUND: While the surgical stages to single ventricle palliation serve to volume-unload the single ventricle (SV), the Glenn and Fontan stages also result in transition from parallel to series circulation. How this transition from parallel to series circulation affects pressure loading of the SV is as yet unreported.

METHODS: Single center retrospective chart review of stage I, II and III cardiac catheterization (CC) and echocardiographic data from 2001-2017. Longitudinal analyses was performed with log-transformed variables. Medication effects were described with Wilcoxon rank-sum testing.

RESULTS: There were 346 total patients (Stage I, n=253; Stage II n=202; Stage III, n=102) who underwent CC at a median age of 4.4 months (Interquartile Range [IQR] 3.4-5.9), 2.9 years (IQR 1.9-4.4) and 7.4 years (IQR 3.5-9.6) respectively. Total Vascular Resistance (TVR) steadily increased as the SV was palliated without significant change to Pulmonary Vascular Resistance (PVR), Systemic Vascular Resistance (PVR) and Cardiac Index (CI). TVR was lower in Stage III patients who were on an anti-hypertensive medication (15.99 vs 21.18 wu x m2, p = 0.051). TVR, PVR, SVR and CI did not correlate with Right Ventricle Fractional Area Change, Left Ventricle Ejection Fraction, or Systemic Valve (SV) or Atrioventricular Valve (AV) function.

CONCLUSIONS: TVR steadily increases with an increasing contribution from SVR as the SV is surgically palliated but did not correlate with echo-based indices of SV function or AV valve function. Further studies are needed to see if modulating TVR can improve outcomes.

114. The Bone Marrow Microenvironment as a Mediator of Resistance to MERTK Inhibition in AML

Vasileiadi, Eleana; Minson, Katherine; Huey, Madeline G.; Wang, Xiaodong; Frye, Stephen V.; Earp, H. Shelton; DeRyckere, Deborah; and Graham, Douglas K.

BACKGROUND: MRX-2843 is a small molecule inhibitor of MERTK, a receptor tyrosine kinase that is a member of the TAM (TYRO-3, AXL, MERTK) family and is aberrantly expressed in acute myeloid leukemia (AML). Treatment with MRX-2843 induces apoptosis in AML cell cultures and results in decreased tumor burden and prolonged survival in cell line and patient-derived murine xenograft models. Although MRX-2843 effectively reduces leukemia in the peripheral blood, there is relative persistence of bone marrow disease burden. **OBJECTIVE:** To investigate the role of the bone marrow stromal niche in providing protection against MERTK inhibition and to determine

whether soluble mediators are sufficient to produce a similar effect. DESIGN/METHOD: AML cell lines were cultured in the presence or absence of Hs27 stromal cells or Hs27 conditioned medium and treated with MRX-2843 or vehicle. Induction of cell cycle alterations in AML cells was determined by flow cytometry. Expression of γ H2AX and pCDC2 was determined by immunoblot. RESULTS: Treatment of AML cell lines with 200nM of MRX-2843 induced significant accumulation of cells in the G2/M phase relative to vehicle-treated cultures ($35.33 \pm 6.82\%$ versus $12.24 \pm 2.26\%$, $p=0.0183$) and decreased pCDC2 levels, indicating increased progression into mitosis. Immunoblot analysis also demonstrated increased expression of γ H2AX upon treatment with MRX-2843, indicating accumulation of DNA damage. In contrast, when leukemia cells were co-cultured with Hs27 stromal cells, treatment with MRX-2843 did not alter cell cycle progression, pCDC2 levels, or γ H2AX expression. Hs27 conditioned medium was not sufficient to provide protection from these effects of treatment with MRX-2843. CONCLUSIONS: When AML cells are in suspension, treatment with MRX-2843 results in accumulation of cells in the G2/M phase of the cell cycle, potentially through induction of DNA damage and a mitotic phase arrest as indicated by increased γ H2AX expression and inhibition of CDC2 phosphorylation. Co-culture with stromal cells abrogated these responses to MRX-2843, but stromal cell conditioned medium was not sufficient to mediate protection. These data support a mechanism by which bone marrow stroma protect against MRX-2843 induced alterations in cell cycle progression and DNA damage and indicate that direct cell-cell contact is required for this effect.

115. Bowel Location Rather than Disease Sub-type Dominates Transcriptomic Heterogeneity in Pediatric IBD

Venkateswaran, Suresh; Marigorta, Urko M.; Denson, Lee A.; Hyams, Jeffrey S.; Gibson, Greg; and Kugathasan, Subra

BACKGROUND: As of 2018, GWAS have identified more than 250 common loci for inflammatory bowel disease (IBD). One of the present challenges is to identify and understand the underlying molecular mechanisms whereby each locus predisposes to IBD. Although gene expression has arisen as a useful tool for these purposes, a better comprehension of the heterogeneity within disease is necessary to better annotate GWAS discoveries. In this regard, datasets profiling patients to explore changes in gene expression across gastrointestinal locations relevant to the disease (e.g. ileum vs colon) are lacking in IBD literature.

AIM: To examine the role of tissue and disease subtypes in pediatric IBD using transcriptomic profiling with RNA-Seq.

METHODS: We analyzed a subset of 153 treatment-naïve samples (118 CD and 35 UC) from the pediatric IBD RISK study that had been profiled with RNA-Seq in both ileal and rectal biopsies. edgeR analyses was used to find genes that are differentially expressed. Linear mixed models with GEMMA were used to find eQTLs and inspect heterogeneity in eQTL effects according to tissue and disease status.

RESULTS: Tissue (ileum vs. rectum) accounts for a larger proportion of the heterogeneity in transcriptomic variation (11.6%), compared to disease sub-type (CD vs. UC; 1%) whereas the interaction between tissue and disease sub-type plays a slightly larger role than sub-type (1.3%). The pairwise comparison between tissue and disease sub-type shows that the transcriptomic signatures in IBD are clustered mainly by tissue types (Ileum and rectum) rather than disease types (CD and UC). Although a fraction of genetic control over gene expression effects vary among sub-groups, most of them are shared regardless of tissue, disease subtypes and inflammation status. The comparative analysis within ileum CD inflamed and non-inflamed region showed that the eQTL effects are not preponderantly exacerbated in the ileum CD inflamed sub-group.

CONCLUSION: Transcriptomic heterogeneity within pediatric IBD clusters mainly by tissue. CD and UC resemble in expression patterns both at the ileal and rectal level. These findings enhance the importance of taking into account the heterogeneity within IBD for the design of experiments profiling molecularly IBD patients.

116. Associating Medication Practices to Unplanned Extubation in Intensive Care Pediatric Patients.

Wang, Yifan; Li, Zihao; Keskinocak, Pinar; and Vats, Atul.

INTRODUCTION: UPE is a multi-factorial problem that leads to complications, higher cost and even death in pediatric care. Current literature focused on identifying risks factors of UPE in adults, while pediatric data for risk factors is limited. There are literatures suggesting that UPE could be related to patient insufficiently sedated or non-standardized weaning protocol. The propose of this study was to focus on a medical surgical PICU population to help identify risk factors for UPE.

METHODS: We adopted a retrospective study in the PICU of CHOA on Egleston campus. The study period is 60 months, from January 2013 to December 2017. Data we used are collected from UPE huddle data, and the download of EMR for all intubated patients including detailed information on sedation received. We used two sample T test and Mann-Kendall trend test for statistical analysis.

RESULTS: During the studied period, a total of 1975 intubation data were collected. Among the total ventilation of 19804 days, 112 UPE cases are documented. The average UPE rate is 0.565 per 100 ventilation days. There have been two sedation medication practice changes since 2014, at January 2015 and July 2017 respectively. We divide our study period accordingly into three phases. Average UPE rate in three phases are 0.35, 0.78 and 0.46 per 100 ventilation days respectively. Specifically, UPE rate decreased from 1.38 cases/100 ventilation days, on average from January to June 2017, to 0.46 cases/100 ventilation days, on average from July 2017 to January 2018. We examined patient demographic characteristics, such as, weight, age, gender, and showed that none of them changed significantly.

CONCLUSIONS: Our results showed that the UPE rate changes are statistically significant between phases. During the second phase, the UPE rate increase is associated with increasing usage of

morphine, precedex and propofol and decreasing usage of hydromorphone, ketamine, and lorazepam (or benzo in total). During the third phase, the UPE decrease is associated with increasing usage of fentanyl (or narco in total) and precedex, and decreasing usage of ketamine. We are currently working on medication usage differences between patients with UPE and without.

117. I2PP2A Compromises p53 and Promotes Tumor Survival of Sonic Hedgehog

Medulloblastoma

Wei, Yun; Maximov, Victor; Morrissy, Sorana; Taylor, Michael D.; Pallas, David C.; and Kenney, Anna M

Medulloblastoma is the most common pediatric brain cancer. Current treatment strategies comprise surgery, radiation and chemotherapy, which generate side effects that severely impact survivors' quality of life. Approximately 30% of medulloblastomas show aberrant Sonic Hedgehog (Shh) pathway activity, thus named as SHH medulloblastoma. The only molecular target for SHH medulloblastoma is Smoothed, an activator of sonic hedgehog signaling. However, resistance occurs in these smoothed inhibitor treated patients, and tumor recurrence is typically fatal. Lack of faithful target of SHH medulloblastoma underscores the need to discover novel oncogenic drivers for this malignant tumor.

The tumor suppressor gene TP53 has been recently recognized as a prognosis marker for SHH medulloblastoma patients. Researchers have shown that five-year overall survival of TP53 wildtype is ~80% and TP53 mutant is ~40%. However, TP53 mutations are only found in ~20% of all cases, and most of them are adult SHH medulloblastoma. Because p53 is not mutated in most of pediatric SHH medulloblastoma, we sought to determine whether its function may be abrogated by other molecular mechanisms. Utilizing transgenic NeuroD2-SmoA1 mouse model, which recapitulates human SHH medulloblastoma in pathology, etiology and molecular profiles, with no p53 mutation reported, we confirmed that p53 can be reactivated by using a drug inhibiting its negative regulator MDM2 (Mouse Double Minute 2). We observed a relatively higher level of p-MDM2 (Ser166) in SmoA1 tumors, an activating site of MDM2 to suppress p53. PP2A, protein phosphatase type 2A could dephosphorylate MDM2 at Ser166. Thus, we hypothesizes the aberrant p-MDM2 might be caused by the dysfunction of PP2A.

As no PP2A subunit mutations have been associated with SHH medulloblastoma, we found that highly elevated I2PP2A (endogenous Inhibitor 2 of PP2A) of SmoA1 tumor suppress p53 function by upregulating p-MDM2. Importantly, this mechanism is conserved in human p53 wild type medulloblastoma cell lines. Taken together, our findings indicate that in p53 wild type Shh medulloblastoma, p53 activity can be modulated by I2PP2A, and they raise the possibility of potentially targeting I2PP2A and other PP2A inhibitors as novel therapeutic approaches, to enhance its activity and improve the effectiveness of current therapeutic regimens.

118. Arthroscopic Management of Pigmented Villonodular Synovitis in Children and Adolescents

Willimon, S. Clifton; Busch, Michael; Schrader, Tim; and Perkins, Crystal

INTRODUCTION Pigmented villonodular synovitis (PVNS) is a benign proliferative synovial disorder most commonly described in adults. The purpose of this study is to describe the presentation and management of a large single-center series of pediatric patients with PVNS of the hip and knee.

METHODS: A retrospective review was performed of consecutive pediatric patients less than 18 years of age who were treated surgically for PVNS of the knee or hip. The primary outcome was disease persistence.

RESULTS: 22 pediatric patients with an average age of 10.9 years (range 2 – 17 years) met inclusion criteria. The knee was affected in 17 patients (77%) and the hip in 5 patients (23%). The average duration of symptoms prior to orthopaedic evaluation was 12.9 months, significantly longer in the knee (16 months) compared to the hip (1 month). Misdiagnosis with a variety of conditions prior to the diagnosis of PVNS was common, occurring in 64% of children.

A total of 34 surgeries were performed in 22 patients. Fourteen patients (64%) were treated with a single surgery. Eight patients required 2 or more surgeries. The majority of procedures were arthroscopic synovectomies (88%). Fourteen patients were noted to have nodular disease, 5 diffuse, and 3 mixed. At average follow-up of 19 months, 22 patients (88%) were considered to be disease-free based on clinical exam and/or follow-up MRI. Two patients (9%) had MRI findings consistent with persistent PVNS of the knee that were stable on serial imaging and without any significant symptoms. No patients with PVNS of the hip had evidence for disease persistence.

CONCLUSION: This case series of pediatric patients with PVNS represents the largest single-center cohort in the literature. Although previously considered a disease of young adults, PVNS should be considered in children with an insidious onset of joint swelling with or without pain. Symptoms may be present for months to years prior to diagnosis. Patients are frequently misdiagnosed, most commonly with rheumatologic diseases, bleeding disorders, or septic arthritis. MRI with gradient echo sequences is the diagnostic imaging study of choice and arthroscopic synovectomy produces good outcomes with low rates of symptomatic disease persistence.

119. Formulation of Nanoparticles to Target Oxytocin to the Brain for the Treatment of Autism *Zaman, Rokon; Damoah, Aboagyewaah; Mulla, Nihal; Murnane, Kevin; and D'Souza, Martin*

INTRODUCTION: The blood brain barrier (BBB) is a highly selective dynamic interface which restricts the movement of most drugs and blood borne molecules into the brain. Although there are a number of large or lipophobic compounds that have shown great potential in the treatment of neurodegenerative diseases, they can not pass the blood brain barrier (BBB). In-order to cross this barrier, endogenous biological mechanisms can be utilized to actively transport these compounds into the brain. An iron binding protein transferrin (Tf) binds to the Transferrin receptors (TfR) expressed on the BBB. Thus Tf-conjugated drug delivery systems (nanoparticles, liposomes and micelles) can improve drug transport across the BBB using receptor mediated transcytosis. In this

study, we examine the development of a transferrin conjugated oxytocin loaded nanoparticulate formulation that can cross the BBB.

METHODS: Transferrin conjugated PLGA based nanoparticles were made following multiple emulsion solvent evaporation method. Particles were characterized in vitro for size, zeta potential, encapsulation efficiency and release profile. The amount of the oxytocin released was determined by oxytocin specific ELISA. In order to determine the brain penetrance of the nanoparticles, transferrin conjugated indocyanine green loaded nanoparticles were injected to mice via intraperitoneal route along with unconjugated nanoparticles with indocyanine green and indocyanine green solution as controls.

RESULTS: The formed nanoparticles were slightly negatively charged (-7.56mV) and the average size was 191.7 nm in diameter. Encapsulation efficiency was 81.4%. The release study demonstrated that about 26% of encapsulated oxytocin was released by day 33. Bio-imaging data showed that nanoparticles crossed the BBB within 30 minutes of the administration of the particles.

CONCLUSION: This study showed that the transferrin conjugated nanoparticulate formulation can cross the blood brain barrier (BBB).

TRANSLATIONAL IMPACT: This nanoparticulate vehicle can potentially be used to target drugs and therapeutic peptides such as oxytocin to the brain in order to treat patients with autism and different neurodegenerative diseases.

120. Early Life Pain Differentially Alters Microglial Expression in the Periaqueductal Gray of Male and Female Rats

Zamor, Jonassie; Hanus, Lauren; Fullerton, Evan; Rubaharan, Myu; Bell, William; and Murphy, Anne

INTRODUCTION: The developing brain is extremely sensitive to exogenous signals, and recent studies suggest that adverse experiences during the perinatal period, including maternal stress, trauma, or infection, may profoundly alter developing neural circuits. We have previously shown that a single inflammatory insult on the day of birth significantly alters opioidergic neural circuits within the midbrain periaqueductal gray (PAG), permanently changing subsequent responses to pain and stress. The activation of microglia, the resident immune cells of the brain, may facilitate these alterations to the stress circuit. However, to date, the impact of early life pain on microglia expression and activation across the life span are unknown.

METHODS: Within 24 hours of birth, male and female rat pups received an injection of the inflammatory agent carrageenan into the right hindpaw or were handled. Tissue was collected at postnatal days (P) 7, 14, 21, 40, or 60 days after birth (P7, P14, P21, P40, P60). The tissue was sliced and stained for proteins specific to microglia using immunohistochemistry. Microglia phenotype was characterized as amoeboid, activated or quiescent based on morphology.

RESULTS: No differences in total microglia in the PAG were observed for early life pain versus control rats. Although rats that received an early life injury had more glial activation in the PAG at P40 than the rats who were handled, the handled rats at P60 showed more microglial activation than the injured rats. This indicates a change in activation as rats increase in age. Similar results in varied microglial activation are expected for P7, P14, and P21.

DISCUSSION: While early life injury does not affect overall microglia cell number in the PAG, it results in a gradual decrease in activation threshold in an age-dependent manner. These results may have significant implications for understanding the mechanisms underlying alterations in pain and stress sensitivity that are observed across the lifespan.

121. ETV6 P214L Mutation Impairs Hematopoietic Stem Cell Function and Perturbs Leukocyte Profiles in a Mouse Model

Zhou, Chengjing; Uluisik, Vizvan; and Porter, Christopher

INTRODUCTION: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer in US and remains the leading cause of cancer death in children. ETV6 is one of the most commonly mutated genes in ALL. Currently the function of ETV6 has been mainly studied in the context of its fusion with RUNX1 and other partners. That cannot assess the function of ETV6 in hematopoiesis and leukemogenesis.

METHODS: A novel mouse model of ETV6 P214L mutation was used in this study. 1) The bone marrow (BM) cells harvested from ETV6 WT (WT) and ETV6 P214L heterozygous (Het) mice were stained with the antibody cocktails to identify hematopoietic progenitor compartments and early B cell fractions. The analysis was accomplished with a LSRII flow cytometry. 2) Bone marrow transplantation (BMT) assay. Whole BM cells from the donor mice, including ETV6 P214L homozygous (HO) were transplanted into X-ray irradiated (5+5 Gy) Rag1^{-/-} mice. Based on received donor cells, the recipients were assigned into seven groups: WT/BoyJ, Het/BoyJ, HO/BOyJ, WT, Het, HO and BoyJ. Donor cell engraftment were monitored by analysis of periphery blood lineage profile with flow cytometry and CBC count.

RESULTS: Our results showed that ETV6 P214L Het slightly increased LT-HSCs (CD48-CD150+CD34-) and their immediate progeny, MPP1s (CD48-CD150+CD34+), while ST-HSCs (CD48+CD150-) remained the same and MPP2s (CD48+CD150+) decreased. These results implied ETV6 may play a significant role in maintaining LT-HSC function. Our BMT assay further confirmed this hypothesis. Mice transplanted with Het and HO BM cells showed slightly decreased total WBCs in periphery blood at 12 and 16 weeks. With Boy J cell (CD45.1) competition, the percentage of Het and HO WBCs (CD45.2) continued decrease, 45% in Het/BoyJ and around 40% in HO/BoyJ at 16 weeks. Interestingly, under competition, the percentage of B cells within CD45.2 cells increased, while percentage of T cells slightly decreased in HO/BoyJ.

CONCLUSION: ETV6 play a significant role in hematopoiesis. ETV6 P214L mutation disturbed LT-HSC function and altered periphery blood WBC profiles.

122. Workflow Analysis of Cardiovascular Imaging Research Core (CIRC) Services: A Research Staff Survey

Zinck, Kelsey; Krupa, Nicole; Sachdeva, Ritu; and Lipinski, Joan

BACKGROUND: The Cardiovascular Imaging Research Core (CIRC) provides cardiovascular imaging for research studies for cardiology and other sub-specialties. We conducted a survey of research staff to determine the factors influencing their experience while using CIRC services. Addressing these factors could help improve CIRC workflow.

METHODS: The survey included research staff demographics, their training with scheduling CIRC studies, the volume and type of research studies performed through CIRC, and their experience with delays in services provided by CIRC. A 5-point Likert scale was used wherever applicable.

RESULTS: Responses were obtained from 26 of 40 (65%) research staff members (88% females, 61.11% with a BS degree, 34.62% with <3 years experience in clinical research). Of the 26 respondents, 58.33% were research coordinators, with 32% of respondents having >5 active studies utilizing one or more imaging studies (echo, MRI or vascular imaging). Of staff that had >3 open or closed studies requiring imaging studies, 44% were >satisfied with their experience with CIRC. Six of the 9 respondents (66%) that enrolled >10 patients a year on average, requiring >1 imaging study, were >satisfied with their experience with CIRC. There was no significant relation between volume of studies or volume of patients and satisfaction with CIRC. Respondents who answered that scheduling was very easy also responded that they were very satisfied with their experience with CIRC. Of the 8 that responded that the CIRC appointments started on time >80 % of the time, 7 were >satisfied with CIRC and 5 of 8 responded that it was easy to schedule appointments with CIRC. How and when staff was trained to schedule with CIRC did not impact their scheduling experience.

CONCLUSIONS: Ease with scheduling patients and avoiding delays during appointments with CIRC appears to positively impact research staff satisfaction. Improving the process for scheduling patients with CIRC through the use of an online form and assuring timely appointments could help improve the workflow and research staff experience.

Index

- Addo, Richard, 45
Adekunle, Oluwaseyi, 6, 47
Agarwal, Maneesha, 66
Aguilera, Dolly, 82
Ahn, Song Ih, 25
Aioub, Allison, 7
Akbulgic, Oguz, 55
Akintoye, Olalade, 87
Alexander, Matthew, 41
Alexander, Neneka, 46
Anderson, Larry, 44
Aplenc, Richard, 60, 61
Arafat, Dalia, 12
Archer, David R., 11
Asmussen, Niels C., 7
Aumann, Waitman, 8
Axelrod, Jed, 67
Bagatell, Rochelle, 60
Bailey, Lynn B, 50
Bajaj, Lotika, 9, 45
Bakshi, Nitya, 75, 76
Baldassano, Robert, 62
Ballengee, Cortney, 18
Bansal, Amit, 9, 10
Baranak, Andrew, 31
Bartlett, Chris M., 31
Baxter, Katherine, 42, 70
Bell, Gregory V., 11
Bell, William, 11, 92
Bennett, Ashley M., 11
Berger, Angela K., 74
Berger, Kiera, 12
Berry, Jameson, 13
Beveridge, Jennifer, 7
Beyersdorf, Jared, 77
Bhatt, Sonal, 24
Bhowmik, Tuhin, 45
Bhuiyan, Taufiqur, 47
Bland, Victoria L, 50
Bloch, Deborah, 13
Bodner, Douglas, 14
Border, William, 34
Boss, Emily, 32, 33
Boyan, Barbara D., 7
Boyle, Brendan, 61
Braithwaite, Kiery, 20
Braz Gomes, Keegan, 15
Braz Gomes, Kimberly, 15
Brown, Chester, 4
Brown, Lou Ann, 36, 73
Bryan, Leah, 42
Buchanan-Perry, Iris, 16
Buckley, Erin M., 52
Burrows, Evanette, 61
Busch, Michael, 20, 67, 68, 69, 90
Bush, Nicole, 70
Caldara, Amber, 19
Caltharp, Shelley, 55
Carter, Alexis, 13, 82
Cash, Thomas, 82
Caspary, Tamara, 71
Castellino, Sharon, 59, 82
Castillo-Ruiz, Alexandra, 15, 43
Caudill, Marie A, 50
Chakravorty, Samya, 12
Chambliss, Christopher, 16
Chan, Melissa, 17
Chanani, Nikhil, 13, 43
Chandler, Joshua D., 17
Chandradevan, Raguraj, 18
Chang, Hua, 41
Chen, Johnny, 78
Chen, Suephy C., 32
Chen, Zhihong, 57
Cheng, Shing Shin, 79
Chenot, Haley, 7
Chitalia, Yash, 79
Cho, Alice, 6, 19
Cho, Hee Cheol, 77
Chonat, Satheesh, 11
Christino, Melissa, 20, 67, 68, 69
Clifton, Matthew, 42
Cochran, Shelton, 41
Conneely, Karen, 23, 81
Conway, Amanda, 8
Corey, Sally, 20
Costo, Megan, 21
Cranmer, Lisa, 21
Crepeau, Rebecca L, 22
Currier, Michael, 53
Curtis, Sarah W., 23
Cutler, David, 81
Damoah, Aboagyewaah, 91
Daniela Fuchs, Claudia, 54
Darby, Trevor, 23, 64
Darden, Truddie, 16
Dasgupta, Soham, 24
Davidson, Kelsey, 69
Davis, Michael, 80
Davis, Robert L, 55
Dawson, Paul, 54, 71
Day, Cheryl, 21
DeGrauw, Ton J, 63
Denham, Megan E., 31
Denson, Lee, 62, 81, 88
DeRyckere, Deborah, 72, 87
Desai, Jaydev, 79
Deshpande, Shriprasad, 24, 45, 84
Devito, Dennis, 67
Dey, Abhinav, 25
Dougan, Jodi, 29
Dreaden, Erik, 3, 47
D'Sa, Sucheta, 9, 57
D'Souza, Martin, 9, 10, 15, 45, 57, 91
Duan, Meixue, 36, 73
Earp, H. Shelton, 87
Easton, George, 66
El-Sayegh, Lydia, 25
Espinoza, Tamara, 51
Fabregas, Jorge, 67
Fain, Brad, 31
Feldman, Ron, 19
Felker, James, 25, 82
Ferdous, Salma, 26
Ferguson, Eric, 34
Fernandez, Natasha, 77
Ferrebee, Courtney, 54
Fialkowski, Allison, 27
Finlayson, Michael, 28
Finn, M.G., 7
Fisher, Brian, 60
Fisher, Susan, 50
Fiss, Alyssa, 28
Flanagan, Jill, 67
Fleming, Jennifer M, 50
Fogel, Mark, 85
Fonseca, Jairo A, 29
Ford, Aiden, 30, 56, 64
Ford, Mandy L, 22
Forger, Nancy G., 15, 43
Foster, Amanda, 31

- François, Sandy, 32
Fritz, Alexa, 80
Frye, Stephen V., 87
Fullerton, Evan, 92
Gaffney, Sierra, 32, 33
Gagaring, Kerstin, 83
Gaitonde, Mansi, 34
Gala, Rikhav, 9, 45
Gamal, Wael, 9
Gardner, Lori, 29
Garren, Mark, 7
Gavegnano, Christina, 35
Gazmararian, Julie, 46
Gearheart, Christy, 29
Gee, Beatrice, 16, 35
Geng, Zhishuai, 7
Gentillon, Cinsley, 36
George, Alex, 80
George, Roshan, 46
Getz, Kelly, 60
Gibbs, Devin, 41
Gibson, Greg, 12, 28, 36, 62, 73, 81, 88
Gilleland Marchak, Jordan, 17
Gillespie, Scott, 21, 42, 66
Go, Young-Mi, 18
Goldsmith, Kelly C., 80, 82
Gonzalez, Mark D., 13
Gonzalez, Renzo, 49
Gore, Russell, 51
Gowda, Charitha, 48
Graham, Douglas K, 72, 82, 87
Greenbaum, V. Jordan, 66
Grewenow, Stephanie, 71
Griffiths, Anne, 61
Grimm, Nathan, 69
Grossniklaus, Hans E, 26
Gruenewald, David, 13
Grundmeier, Robert, 61
Guberman, Jaime, 74
Guglani, Lokesh, 18, 37
Ha, Binh, 44
Hambardzumyan, Dolores, 57
Hams, Jeffrey S., 81
Hanus, Lauren, 38, 92
Harper, Elizabeth, 66
Harris, Frank, 73
Harris, Jason, 47
Hartert, Tina, 44
Hashemi, Sassan, 38
Haston, Julia, 39
Hausman, Dorothy, 50
He, Xi, 40
Hegde, Madhuri, 12
Henry, Curtis, 29
Herzog, Mackenzie, 68
Hightower, Rylie, 41
Hillyer, Margot, 42
Hoffenberg, Sara, 58
Hoffiz, Yarely, 15, 43
Hoffman, Ryan, 43
Holthausen, David, 78
Honegger, Jonathan, 48
Horati, Hamed, 17
Hovell, Candice, 25
Huang Yuan-Shung, 60
Huang, Xiqin, 27
Huey, Madeline G, 72, 87
Huey, Madeline G., 87
Hyams, Jeffrey, 18, 61, 88
Hyams, Jeffrey S., 88
Ibrahim, Amenah, 51
Islam, Muhymin, 3
Jacob, Joshy, 78
Jacobs, Andrew J., 15
Jadhaio, Samadhan, 44
Janssens, Hettie M., 18
Jaspan, Heather, 21
Jeffries, Lynn, 28
Jen, Emily, 60
Jerris, Robert, 55
Jha, Rajneesh, 36, 45, 73
John-Stewart, Grace, 21
Joiner, Clinton, 11, 35
Jones, Dean P., 18
Jones, Kenneth L., 86
Jones, Rheinallt, 23, 64
Jones, Shannon, 34
Jones, Warren, 30, 56, 58, 64
Jordan, Dorothy, 27
Joshi, Devyani, 45
Jun, Kong, 47
Kagina, Ben, 21
Kaiser, Eileen, 21
Kamel, Margret, 46
Kang, Sang-Moo, 15, 57
Kanter, Kirk, 85
Kasi, Ajay, 37
Kauffman, Robert, 6, 47
Kauwell, Gail PA, 50
Keljo, David, 62
Kelleman, Michael, 73
Keller, Frank, 59
Kelvin, James, 47
Kennedy, Samuel, 48
Kenney, Anna, 25, 57, 90
Keskinocak, Pinar, 40, 89
Khemani, Kirshma, 75, 76
Kilaru, Varun, 81
Kilgore, Matthew B., 17
Kim, Baek, 35
Kim, Jenny, 7
Kim, Yongtae, 25
Kim, Yun-Soung, 53
Kinsey, Joshua, 49
Kirschman, Jung Hwa, 49
Klaiman, Cheryl, 56, 58
Klin, Ami, 30, 56, 58, 64
Knight, Anna, 50, 70
Knipp, Gregory, 45
Kosoris, Nicole, 51
Kowalczyk, Andrew, 19
Krishnamurti, Lakshmanan, 75, 76
Krupa, Nicole, 94
Kubanek, Julia, 83
Kugathasan, Logan, 62
Kugathasan, Subra, 18, 62, 81, 88
Kumar, Vishnu, 44
Kunkel, Louis, 41
Lai, Kristina, 51
Lam, Wilbur, 52
Landesman, Yosef, 41
Lane, Peter A, 51
LaPlaca, Michelle, 51
Lavau, Catherine, 8
Lawley, Leslie P., 32
Lee, Christopher, 41
Lee, Seung Yup, 52
Lee, Song-Hee, 78
Lee, Sujin, 53
Lee, Yongkuk, 53
Lee, Yoo Joo, 80
LeLeiko, Neal, 62
Lense, Miriam, 30, 56, 64
Li, Jianing, 54
Li, Yimei, 60
Li, Zihao, 89
Lin, Zhao, 7
Lipinski, Joan, 94
Liu, Brian, 51
Liu, H. Ken, 18
Liu, Jingbo, 25
Liu, Mingli, 16
Luo, Liping, 23
MacDonald, Heather, 55

- MacDonald, Tobey, 25, 82
Mack, David, 61
Mahajan, Ruhi, 55
Maher, Kevin, 45, 53
Mainou, Bernardo, 13, 74
Malhotra, Anshu, 25
Malone, Tamika, 71
Malysheva, Olga, 50
Maner-Smith, Kristal, 64
Marcus, Michele, 23
Margaroli, Camilla, 17
Marigorta, Urko, 61, 81, 88
Marin, Mariana, 49
Markert, Sarah, 30, 56, 64
Markowitz, James, 62
Masino, Aaron, 61
Matsuo, Kumiyo, 87
Maximov, Victor, 57, 90
Maxwell, Joshua, 73
McCain, Robyn, 45
McCallum, Susan, 63
McCarthy, Ian, 70
McClure, Michael J., 7
McCracken, Courtney, 34, 44, 46, 66
McElroy, Anita, 39
McNamara, Case, 83
Medda, Alessio, 51
Medrano-Trochez, Camila, 28
Melikian, Gregory, 49
Mener, Amanda, 11
Menon, Ipshita, 57
Mertens, Ann, 17, 59
Micheletti, Megan, 58
Michelfelder, Erik, 34
Miller, Lane, 59
Miller, Tamara, 60, 61
Minson, Katherine, 72, 87
Mitchell, Sarah, 82
Mo, Angela, 61
Mondal, Kajari, 18, 81
Moore, Martin, 53
Morris, Claudia, 73
Morrissy, Sorana, 90
Mosley, Morgan, 15
Mosley, Trenell, 62
Mulla, Nihal, 91
Mulle, Jennifer, 71
Murnane, Kevin, 91
Murphy, Anne, 11, 38, 92
Murphy, Joshua, 67
Naik, Kushal B, 63
Naudin, Crystal, 64
Nduba, Videlis, 21
Nickerson, John M., 26
Nielsen, Jørgen G, 37
Njuguna, Irene, 21
Novack, Jack, 43
Okou, David T., 81
Olmstead, Jack, 30, 56, 64
Olmstead, Jack., 64
Olszewski, Dana, 67
Onyekaba, John, 16
Ortlund Eric, 64
O'Reilly-Shah, Vikas, 65, 66
Owens, Joshua, 23
Pachura, Kimberly, 54, 71
Palacios, Gustavo, 39
Pallas, David C., 90
Park, Hea Jin, 50
Park, Kihan, 79
Park, Sunita, 82
Parks, W. James, 38
Parsons, Will, 1
Patel, Bhavesh, 87
Patel, Seema R., 11
Pauly, Melinda, 82
Payne, Aimee, 19
Peckham, Sheri-Ann, 66
Pedersen, Knud, 37
Peng, Limin, 18
Perdue, Lacey, 47
Perkins, Crystal, 20, 67, 68, 69, 90
Petit, Christopher, 87
Pilkay, Stefanie, 70
Piper, Kaitlin, 70
Platt, Manu, 80
Pollak, Rebecca, 71
Popescu, Carmen, 45
Porter, Christopher, 29, 82, 86, 93
Pratt, Catherine, 39
Prieto, Karla, 39
Przepiorka, Donna, 60
Purcell, Ryan, 71
Qadri, Firdausi, 47
Qiu, Annie, 72
Qu, Cheng-Kui, 36
Rabe, Jennifer, 29
Rampoldi, Antonio, 73
Ran, Nina, 19
Rao, Anuradha, 54
Raol, Nikhila, 32, 33
Raval, Mehul, 42, 70
Reyes, Loretta, 73
Richardson, Tatayana, 16
Roberts, Leah, 55
Robinson, M. Hope, 57
Rodriguez Stewart, Roxana M., 74
Rogers, Beverly, 55
Rosa, Gisselle, 50
Rosh, Joel, 62
Ross, Diana, 75, 76
Rossignac, Jarek, 85
Rostad, Bradley, 20
Rostad, Christina, 39
Royal, Charmaine, 1
Rubaharan, Myu, 11, 92
Rutkowski, Timothy, 71
Ryan, Edward, 47
Sachdeva, Ritu, 34, 87, 94
Saeedi, Bejan, 23
Sallee III, Denver, 38
Sanders, Bharat, 52
Santangelo, Philip, 77
Sasaki, Jodie, 32
Sasser, Loren, 21
Sauer, Cary, 18
Saulnier, Celine, 58
Sayegh, Michael, 77
Schaafsma, Belinda, 68
Scheniderjaan, Matt, 25
Schmitz, Michael, 67
Scholte, Bob J., 18
Schrader, Tim, 67, 90
Schwartz, Zvi, 7
Schwebel, David C., 84
Sei, Yoshitaka, 25
Seif Alix, 60
Serpooshan, Vahid, 5
Sha, Ying, 78, 84
Shaban-Nejad, Arash, 55
Shane, Andi, 13, 39
Shanmugam, Nithya S., 57
Shartouny, Jessica, 78
Sheng, Jun, 79
Shenoy, Sreekala, 12
Shepard, Caitlin, 35
Shim, Jenny, 80
Shin, Eun, 55
Shoja-Taheri, Farnaz, 80
Shultz, Sarah, 30, 31, 58, 64
Simon, Harold K., 66
Singh, Monalisa, 73
Sinha, Cynthia, 75, 76
Slesnick, Timothy, 38, 85

- Smith, Alicia, 23, 50, 70, 81
Smith, Sharon, 25
Smith, Shelby, 32
Sokol, Joel, 40
Sokolow, Andrew, 50
Somnini, Hari, 18, 81
Spinazzola, Janelle, 41
Stano, Armando, 49
Stapel-Wax, Jennifer, 21
Stark, Megan, 24
Starks, Miah, 37
Stenger, Elizabeth, 28
Stiles, Jonathan, 16
Stowell, Sean R., 11
Summers, Ryan J., 82
Swanson, Erik, 51
Sweeney-Jones, Anne Marie, 83
Tamir, Sharon, 41
Tang, Kerri, 84
Tang, Roderick, 53
Taurone, Alexander J., 18
Taylor, Michael D., 90
Terrell, Metrecia L., 23
Thomas, Jasmine, 27
Thompson, Charlie, 25
Tong, Li, 78, 84
Trauner, Michael, 54
Travers, Curtis, 42, 87
Trusty, Phillip, 85
Tsang, Vivian W. L., 25
- Tylavsky, Frances, 70
Ubale, Ruhi, 45
Uluisik, Rizvan C., 86
Uluisik, Vizvan, 93
Uppal, Karan, 18
Vaiyani, Danish, 87
van Linden, Annemie A., 86
Vasileiadi, Eleana, 87
Vasquez, Abigail, 15
Vats, Atul., 89
Veltman, Mieke, 17
Venkataraman, Sujatha, 86
Venkateswaran, Suresh, 18, 81, 88
Vibhakar, Rajeev, 86
Virtue, Theodore, 25
Walters, Thomas, 62
Wamalwa, Dalton, 21
Wang, Chuan-En, 62
Wang, May, 43, 78, 84
Wang, Xiaodong, 87
Wang, Xuefeng, 79
Wang, Yifan, 89
Wasilewski-Masker, Karen, 17
Wechsler, Dan, 8, 82
Wei, Yun, 57, 90
Wei, Zhenglun, 85
Weiner, Daniel J., 37
Widrick, Jeffrey, 41
Wilcox, William, 62
Wiley, Michael, 39
- Wilkerson, Alexandria, 46
Williamson, Rebecca, 17
Willimon, S. Clifton, 20, 67, 68, 69, 90
Winterberg, Pamela, 73
Withycombe, Janice, 27
Wolfson, David, 77
Woods, William G., 59
Wrammert, Jens, 6, 19, 47
Wright, David, 51
Wu, Qingling, 73
Wu, Xianfu, 10
Wynn, Grace, 54, 71
Xie, Yao, 40
Xu, Chunhui, 36, 45, 73
Yehya, Nadir, 61
Yeo, Woon-Hong, 53
Yoganathan, Ajit, 85
Yoo, Young Moo (Daniel), 65
Yu, Wen-Mei, 36
Zaman, Rokon, 91
Zamor, Jonassie, 92
Zhang, Xiaoyu, 73
Zheng, Leo, 7
Zhou, Chengjing, 93
Zinck, Kelsey, 94
Zmitrovich, April, 66
Zughaier, Susu, 9
Zwick, Michael, 49, 62, 71