Abstracts

Oral Presentation Abstracts

In order of presentation

Longitudinal Development of Social Visual Engagement in Infants Later Diagnosed with ASD
Olson, Lindsay; Klin, Ami; Shultz, Sarah; Jones, Warren

Background: From birth, typically-developing infants preferentially attend to the social signals of their caregivers such as eye gaze (Haith et al., 1977). Recent findings from our laboratory revealed that infants later diagnosed with autism (N = 11) exhibit decline in eye fixation from 2 to 24-months of age whereas typically developing (N = 25) infants show an increase in eye fixation (Jones & Klin, 2013). These findings represent the earliest known indicators of social disability in infancy. If confirmed in a larger sample, findings will inform both early identification and intervention for individuals who have ASD prior to the emergence of the symptoms of ASD. Objectives: Measure growth charts of social visual engagement from 2-24 months infants TD infants and infants who developed ASD. Methods: 103 infants were enrolled as risk-based cohorts: N = 51 at low risk (31-males) and N = 52 high-risk infants (33 males). Diagnostic status was ascertained at 36 months. Of the HR sample, 13 infants received a diagnosis of ASD, 10 BAP, and 29 showed typical outcomes. Infants were shown scenes of naturalistic caregiver interaction as in Jones & Klin (2013). Videos were coded for eyes, mouth, body, and object regions. Eye tracking data collected at months-2, 3, 4, 5, 6, 9, 12, 15, 18, and 24 were used to calculate percent of fixation time to each region. Longitudinal analyses for visual fixation were conducted by principal analysis by conditional expectation (PACE). Longitudinal looking profiles for cohort 2 were compared cohort 1 (2013) for eyes, mouth, body, and object regions. Cohorts 1 and 2 were combined for comparisons between TD and ASD infants. Results: Between group analyses of 2–to-24 month longitudinal looking curves by functional ANOVA show no significant differences in eye, mouth, body, or object fixation between cohort 1 and cohort 2 for TD or ASD infants (all $P > .05$). Cohorts 1 and 2 were combined for analyses between TD and ASD infants. Between-group comparisons revealed significant differences in eye ($F = 4.7553$; $p = 0.0335$) and body ($F = 11.6684$, $p = 0.0012$) but not mouth ($F = 0.0113$; $p = 0.9157$), or object ($F = 1.2438$; $p = 0.2696$) fixation. Conclusions: Results replicate earlier findings that infants who develop ASD show a decline in eye gaze from 2-to-24 months. Results confirm early indications of social disability in infants who develop ASD.

Drug Screening in Zebrafish to Identify Corrective Compounds for Muscular Dystrophies
Alexander, Matthew; Gibbs, Devin; Spinazzola, Janelle; Mead, Lillian; Kunkel, Louis

Zebrafish (Danio rerio) are a powerful tool to study human diseases and identify novel corrective drug compounds. Additionally, zebrafish contain 70% of the same genes as humans, have a low maintenance cost, ex vivo development, and can rapidly uptake small molecule drug compounds up to 7 days post-fertilization (dpf). Zebrafish are an attractive model for muscular dystrophy research due to their evolutionary conservation of all known muscular dystrophy causative genes. We have performed targeted and unbiased library drug screens on zebrafish models of muscular dystrophy, specifically the sapje/(dystrophin), patchytail/(dystroglycan), and candyfloss/(laminin alpha 2) mutant zebrafish models of human muscular dystrophies. From these library drug screens, we have identified a set of common drug compounds that correct muscle wasting phenotypes and extend the lifespan of these muscle mutant zebrafish. The lead compounds identified from these screens had significant effects on HMOX1/cGMP

signaling pathways. Secondary screening in mouse models of muscular dystrophy showed that these compounds improved muscular dystrophy disease pathology by blocking ischemia, reducing fibrosis, and improving overall muscle performance in functional assays. Several of these compounds identified from these zebrafish muscular dystrophy screens are currently FDA-approved compounds for other uses, and can be repurposed for muscular dystrophy therapeutics. Our approach demonstrates that it is possible to identify a corrective drug compound in zebrafish that leads to novel therapeutic entry points in human neuromuscular diseases.

HIV Pediatric Cure: a Rhesus Macaque Model

Mavigner, Maud; Habib, Jakob; Schinazi, Ray; Geleziunas, Romas; Sodora, Don; Estes, Jacob; Silvestri, Guido; Chahroudi, Ann

Of the estimated 35.3 million people worldwide infected by HIV, 3.2 million are children. While antiretroviral therapy (ART) greatly reduces the mortality and morbidity of HIV infection, viral rebound quickly ensues if drugs are stopped due to the persistence of the virus in reservoirs. A fundamental barrier to an HIV cure is our incomplete understanding of the cellular and anatomic reservoir. Given the specific characteristics of the infant immune system, pediatric studies of HIV reservoir are of critical importance. In this study, four infant rhesus macaques (RM) were orally infected with SIVmac251 at 19-20 weeks of age and treated with a potent 3 drug ART regimen initiated 35 days post-infection. Plasma SIVmac251 RNA and cell-associated DNA levels were measured by real-time PCR and immunological parameters were monitored by flow cytometry and immunohistochemistry. Tissue levels of SIV RNA and DNA were quantified by RNA/DNAscope. The frequencies of peripheral memory CD4+ T-cell subsets (central, transitional and effector) pre-infection were significantly lower in infants RM as compared to a group of adult RM (5.1-7.3 years old) emphasizing the need of a specific pediatric model to study SIV reservoirs. Interestingly, the total absolute number of peripheral long-lived CD4+ T memory stem cells (TSCM) per animal was lower in infants than in adults, possibly limiting the size of the reservoir. Plasma SIVmac251 RNA level declined of 2.84 log (2.81-3.2) over the first 2 weeks of ART, reaching undetectable levels (< 60 copies/ml) in 2/4 RM after 17 weeks of ART. Elective necropsy was performed after 6 months of ART. Immunohistochemistry analyses of TNF-α and Mx-1 showed very low level of inflammation in the lymph nodes, spleen, ileum and brain of the ART-treated infant RM. SIV RNAscope showed reduced levels of SIV RNA in the spleen and lymph nodes but not in the brain of the ART-treated infant RM as compared to viremic infant RM. Finally, to allow a comprehensive analysis of the sites of viral persistence, viral DNA was quantified by PCR in sorted memory CD4+ T cell subsets. This study establishes a model of pediatric SIV infection and viral suppression under ART in infant RM unavailable to date. The model provides an experimental in vivo platform to study SIV reservoirs and test cure strategies in the period of infancy.

Development of a Novel Aptamer Screening Platform to Identify High Affinity Ligands for Non-Nucleotide Targets

Milam, Valeria; Tapp, Maeling; Dennis, Patrick; Naik, Rajesh

Aptamers are single-stranded oligonucleotide sequences that exhibit high affinity and specificity for a particular non-nucleotide target including, but not limited to small biomolecules, proteins, and even whole

cells. Aptamers are conventionally identified using a multi-round screening approach called "Systematic Evolution of Ligands by EXponential enrichment" (SELEX) in which a pool of billions of candidate random sequences is continuously enriched with amplified copies of “winning” sequences or adsorbates from prior selection rounds. While SELEX has revolutionized the discovery of numerous aptamers, we have developed a non-SELEX screening approach we call CompELS (Competition Enhanced Ligand Selection) to identify single-stranded DNA aptamers in a much faster, but reliable manner. One of the key differences in our competition-based aptamer screening approach is the elimination of intermittent, time-intensive elution and amplification steps of random sequences that (1) can introduce undesired PCR side products (e.g. partially elongated duplexes) into the candidate sequence pool and (2) bias the candidate pool towards early selection round winners that may simply outnumber higher affinity aptamer candidates. To develop this novel CompELS screening approach, we chose a model non-nucleotide target, namely gold, in a variety of forms (e.g. planar gold, gold nanospheres, gold nanorods). In addition to several advantages as a target material (e.g. chemically inert surface; ease of separation/recovery during screening; negligible binding affinity to oligonucleotide duplexes, etc.), our choice of gold-based targets allows for useful comparative studies of aptamers selected for targets of identical chemical composition, but possessing distinct structural features. Following our aptamer screening and selection, we then evaluate candidate aptamer sequences to identify any patterns or “families” sharing primary or predicted secondary structure features. Lastly, we rank our aptamer sequences in terms of their frequency as a bound species using a high throughput sequencing method known as deep sequencing or next generation sequencing. In ongoing and future work, we continue to adapt our unconventional, but flexible aptamer screening approach to hopefully enable faster, easier, yet reliable aptamer identification for a rich range of biological targets including pharmaceuticals, dynamic proteins, and whole cells for numerous therapeutic and diagnostic applications.

Rapid-Fire and Poster Presentation Abstracts

Rapid-fire presenters highlighted in blue

101. Exercise Clearance for Young Athletes: Is Exercise Stress Echocardiography a Useful Tool?
Allen, Nicole; Friedman, Heather; Stark, Megan; Sachdeva, Ritu; Border, William

Background: When children present with chest pain during exercise, parental and clinician concerns often result in limitations from physical activity. Although the first response, it may not be appropriate in every case and could result in promotion of a preventable sedentary lifestyle. Exercise stress echocardiography (ESE) is an under-utilized technique in pediatrics that is useful to detect cardiovascular abnormalities and can also be used to help clear patients for exercise and other physical activities. Our center saw patients who were athletes or wished to be cleared for physical activity and had ESEs ordered by their cardiologists. We aimed to present the practicality of ESEs in the pediatric population for determining the appropriate level of exercise in young athletes. Methods: All patients who underwent a clinically indicated ESE at our institution between 2011 and 2015 were reviewed. Patients who were identified as athletes based on medical notes with an indication for ESE including chest pain, chest pain with exercise or syncope were included. Patients were placed in categories based on their permitted physical activity levels pre- and post ESE as determined by the ordering cardiologist. Categories included normal and abnormal test results, further classified by change in management as: unrestricted from sports, restricted

from sports, surgery recommended, medical management change or continue current management. Results: Approximately 75 (20%) of the patients were identified as athletes based on the aforementioned criteria. 44 (59%) children continued their current physical activity management, 24 (32%) received full exercise clearance, 5 (7%) were restricted from some physical activity, and 1 (1%) had surgery recommended. One patient had missing data. Of these patients, 15 (20%) had abnormal test results while 60 (80%) had normal results. In the abnormal group, 7 (47%) became restricted, had a medical management change or were recommended for surgery. In the normal group, 21 (35%) became unrestricted (full exercise clearance). The physical activity status permitted was changed in 31 (41%) of the 75 patients as a result of ESE. Conclusion: ESE is a valuable tool that reveals structural abnormalities and other potentially dangerous conditions which can be missed in a child evaluated at rest. While an abnormal result may reveal serious defects, including hypertrophic cardiomyopathy which is associated with sudden cardiac death, a normal result can mean exercise clearance and a healthy child. There is utility in ESE because it provides information regarding a patient's ability to maintain or regain an active lifestyle.

102. Development and Automation of a Self-Contained CD4 Enumeration Strategy to Inform HIV Infection and Reduce Mother-to-Child Transmission

Bauer, Westley; Scherr, Thomas; Conrad, Joseph; Haselton, Fredrick; Wright, David

The mother-to-child transmission of HIV is responsible for 90% of children who become infected with HIV. The transmission of HIV can occur during pregnancy, labor, childbirth and breastfeeding. According to the World Health Organization (WHO), the probability of HIV passing from an infected mother that is not undergoing treatment to her child is estimated to be as high as 45%. However, if mothers living with HIV are adhering to antiretroviral treatment and other effective interventions the mother-to-child transmission rate can be reduced to below 5%. Therefore, it is imperative that mothers infected with HIV be diagnosed and undergo treatment before, during, and following pregnancy to prevent transmission to their children. The global burden of the HIV disease remains in low and middle-income countries where medical diagnosis and care is often difficult to access. As so, there is a major need for clinical diagnostics to be used at the point of care in low resource settings for HIV diagnoses. CD4 T-cell counts are commonly used as a surrogate for immune function and are an essential diagnostic employed in HIV disease management. Specifically, WHO guidelines suggest the onset of HIV treatment with patients having below 350 CD4 T-cells/μL of peripheral blood. Our research group has developed and automated an innovative approach to isolate, concentrate, and enumerate CD4 cells employing immunomagnetic separation in conjunction with immunoenzymatic detection in a self-contained cassette. The one-touch automation of this technology gives it great promise to function at point of care without the need of a skilled technician because it reduces the assay to 4 simple steps: transferring a 100 μL whole blood sample into a sample tube, mixing the sample tube, loading the sample into a premade self-contained cassette, mounting the cassette onto the device and pushing a button. The entire system allows for a current limit of detection of 110 CD4 T-cells/μL of peripheral blood with an overall assay time of 35 min.

103. Screening For Critical Congenital Heart Disease: What's the Real Impact?
Bhatia, Anisha; Reddy, Pooja K.; Kelleman, Michael S.; Kochilas, Lazaros K.; Oster, Matthew E.

Background: The goal of newborn screening for critical congenital heart disease (CCHD) using pulse oximetry is to reduce overall mortality and morbidity. However, whether this goal has been realized is unclear. Objective: Our objective was to compare the association of timing of diagnosis of CCHD with in-hospital mortality and post-operative length of stay (LOS). Design/Methods: We performed a retrospective cohort study of infants with CCHD who underwent surgery at a single tertiary center between 8/2010-8/2015 in Georgia, a state in which CCHD screening was added to the newborn screening panel in July 2014. Timing of diagnosis was defined as early (prenatal or symptomatic prior to 24 hours of age), screening (detected via pulse oximetry screening at or near 24 hours of age), or late (symptomatic after 24 hours of age). We developed a logistic regression model to compare in-hospital mortality and a generalized linear model for log-transformed post-operative LOS, adjusting for age at surgery and Society of Thoracic Surgery-European Association for Cardio-Thoracic Surgery (STAT) Congenital Heart Surgery Mortality score. Results: Of 820 infants, 21 were detected via screening and 124 were detected late (3 of whom initially passed screening). Infants detected via screening were similar to those detected early or late with respect to sex and race/ethnicity. While infants detected early or late had higher adjusted odds of postoperative mortality, these findings did not reach statistical significance [OR for early versus screening: 3.06 (0.40 – 23.42), p=0.28, OR for late versus screening 1.78 (0.18 – 17.76), p=0.62). Infants detected via screening had a shorter postoperative LOS as compared to those detected earlier, but similar LOS as those detected late [7.1 days, 95% confidence interval (CI) 5.1-9.8 vs. 10.2 days, 95% CI 9.6-10.8; p=0.02]. Conclusions: In our early experience with CCHD screening, we have not yet been able to detect any improvements in surgical mortality or postoperative LOS for those detected via screening versus those detected late. Early detection of lesions was associated with longer LOS and poorer outcomes compared to those detected late or by screening. This likely reflects the more severe physiologic manifestations of conditions detected prenatally, resulting in development of symptoms within the first 24 hours of life.

104. Factors Associated with Early Termination of Delayed Cord Clamping in Very Low Birth Weight Infants
Bhatia, Anisha; Kane, Andrea F; Shane, Andi L.; Arluck, Jessica C.; Denning, Patricia W.; Patel, Ravi M.

Background: Delayed cord clamping (DCC) refers to the practice of postponing surgical clamping of the umbilical cord for a minimum of 30-60 seconds following delivery to provide an infant additional placental blood transfusion. Meta-analyses of randomized trials have shown DCC results in improved outcomes in preterm infants. However, some infants receiving DCC may need immediate resuscitation, leading to termination of DCC before goal duration of 60 seconds. We evaluated the frequency of early termination of DCC and associated factors in very low birth weight infants (VLBW) undergoing attempts at placental transfusion. Methods: We performed a retrospective observational cohort study of VLBW infants receiving DCC at a single center from 7/2013-7/2015. We included infants <1500 grams at birth who underwent any attempt at DCC. We excluded infants with major congenital anomalies. We evaluated the duration of DCC and used logistic regression models to identify risk factors for early termination. We compared short term physiologic and clinical outcomes between infants receiving complete (60 seconds) versus early termination (< 60 seconds) of DCC. Results: A total of 92 VLBW infants received DCC. Twenty-three

percent (95% CI 15-33%) of infants underwent early termination before 60 seconds, receiving a median of 30 seconds of DCC. Factors associated with early termination were a low 1 minute Apgar score (OR 1.43 per 1 point decrease, 95% CI 1.15-1.78) and delivery by emergent caesarean (OR 30.0, 2.94-307.0), compared to planned cesarean (OR 1.11, 95% CI 0.34-3.62) and vaginal delivery (reference). There was no association with early termination and birthweight (P=0.16), small for gestational age (P=0.82) or multiple gestation (P=0.24). Infants with early DCC termination, compared to complete DCC, had a higher admission blood glucose (72 mg/dL vs. 51 mg/dL; P=0.02) but lower initial hemoglobin (14.2 g/dL vs. 15.2 g/dL; P=0.03) and mean blood pressure (37 mm Hg vs. 42 mm Hg; P=0.03). We found no differences in pH, pCO2, FiO2, and temperature between groups upon admission to the neonatal intensive care unit. In addition, we detected no difference in lowest hemoglobin in the first month of life or mortality between groups. Conclusions: In this study, the majority of infants in whom DCC was attempted received 60 seconds of placental transfusion. However, one-fourth of infants in our study required early termination and alternative strategies, particularly for infants delivered by emergent cesarean, may be necessary to increase the success rates of placental transfusion.

105. Comparison of Long-Term Memory in One versus Two Dose IPV Schedule in Rhesus Macaque Model

Bhaumik, Siddhartha; Kulkarni, Raveendra; Weldon, Will; Oberste, Steven; Pallansch, Mark; Orenstein, Walter; Villinger, Francois; Kaja, Murali

Switching from oral polio vaccine (OPV) to an inactivated polio vaccine (IPV)-only vaccination strategy post-wild type poliovirus (WPV) eradication is necessary to insure against potential risks for return of polio (either from reintroduction of wild viruses or from circulating vaccine-derived polioviruses); but this switch is not easy in low income/resource-limited countries. The Strategic Advisory Group of Experts (SAGE) has recommended that all OPV-using countries implement at least one dose of IPV in their routine immunization programs. However, experience with a one-dose schedule of IPV alone is very limited especially with regard to duration of immunity. Most of the industrialized world uses 4 or 5 doses of IPV. However, such a schedule is problematic in resource-limited/low-income countries; mostly due to prohibitive costs of IPV manufacture, limited manufacturing capacity, issues related to practicality of compliance with a 4- or 5-dose schedule to ensure repeated vaccinations, and a lack of understanding of correlates mediating long term protection induced by IPV. Our study addresses a potential way (s) to determine long-term immunity after fewer doses to overcome this problem. We characterized immune response (e.g., humoral antibodies, memory B cells, and their duration of persistence) following one or two doses of IPV in rhesus macaques. Our result clearly shows that while one dose of IPV induces long-lasting priming effect, a booster is required to sustain and improve the circulating humoral antibodies and memory B cells. Additionally we observed a dose sparing effect of IPV when administered via the intradermal route. These results have major implications for evidence based cost-effective approaches for introduction of IPV in underdeveloped and developing countries and provide insurance against re-emergence of mutant polio strains after OPV termination.

106. Gaze Aversion and Self-Soothing at 3-Months in Infants with ASD: An Exploration of Differences in Self-Regulation

Bradshaw, Jessica; Saulnier, Celine; Jones, Warren; Klin, Ami

Self-regulation in the face of highly stimulating or distressing events is the primary developmental task for 2- to 6-months-old infants. Effective self-regulation and self-soothing during social interactions allows for sustained alertness, enhanced learning opportunities, and the development of fluid, coordinated parent-infant interactions. 3-month-old infants use gaze aversion and hand-to-mouth to facilitate self-regulation. Little is known about how these regulatory capacities at 3-months may promote later social-communicative abilities for infants who are later diagnosed with autism spectrum disorder (ASD). Understanding the form of self-regulatory capacities in early infancy and the function they serve for later social development will illuminate novel developmental pathways for the emergence of social-communication and development of ASD. The goal of the current study is to investigate self-regulatory strategies used by infants with ASD and typically developing (TD) infants during parent-infant interactions and to associate these patterns with later developing social-communication skills. Participants included 14 infants who were later diagnosed with ASD and 23 typically developing infants. At 3-months infants participated in a 30-second parent-infant interaction; parents were not touching or holding their infant, ensuring the absence of potential external regulatory mechanisms. Parent-infant interactions were coded for the occurrence of two self-regulatory behaviors: gaze aversion and oral self-soothing, or “hand-to-mouth”. At 12-months, the Communication and Symbolic Behavior Scales (CSBS) was administered and the Social subscales were included as a measure of social-communicative ability. Results revealed that 3-month-old infants later diagnosed with ASD spent less time using oral self-soothing (“hand-to-mouth”) and significantly less time using gaze aversion ($p<.05$) as a self-regulatory strategy during parent-infant interactions. There was also a significant association between the use of gaze aversion during parent-infant interactions at 3-months and the social and symbolic composites of the CSBS at 12-months. This study provides initial results indicating significantly different use of self-regulatory strategies for infants later diagnosed with ASD and relationships to later social-communicative outcome.

107. Multimodality Therapy with Ionizing Radiation and a Small Molecule WIP1 Inhibitor Suppresses Growth of Patient-Derived Models of DIPG

Brown, Briana D.; Akamandisa, Mwangala P.; Wen, Jing; Hambardzumyan, Dolores; Castellino, Robert;

Diffuse Intrinsic Pontine Glioma (DIPG) is aggressive, difficult to treat, and the main cause of brain tumor-related deaths in children. These tumors arise in the pons and comprise more than 80% of pediatric brain stem tumors. Little to no improvement in prognosis has been made in over three decades. Currently, patients survive an average of nine to ten months and the overall survival rate with this disease is less than 10%. Due to the infiltrative nature of this tumor, surgical resection is impossible and radiation (IR) therapy only provides palliation. Additionally, alternative treatments such as chemotherapy provide no additional benefit. Identification of molecularly-targeted therapies for the treatment of DIPG is essential to improve treatment response and overall prognosis. WIP1 is a protein phosphatase known to act as a proto-oncogene when overexpressed in various cancers, including the high-grade pediatric brain tumor, medulloblastoma. We hypothesize that WIP1 truncating mutations enhance inactivation of proteins essential to DNA damage response, including γH2AX, CHK2, and TP53, which results in radiation resistance and DIPG progression. Previous research has shown that C-terminal truncating mutations in

WIP1 promote increased protein stability and inhibition of DNA damage responses in osteosarcoma and colorectal cancer cell lines. While WIP1 mutations are known to exist in up to 25% of DIPGs, the role these mutations play in DIPG progression is unknown. Our lab has shown that transduction of C-terminal WIP1 mutations into patient-derived DIPG cells inactivates DNA damage response effectors P53 and γH2AX, and increases AKT phosphorylation in response to IR. These C-terminal WIP1 mutations also significantly increased proliferation. Engraftment of WIP1-mutant DIPG patient-derived neurospheres onto murine brain slices promoted outgrowth of DIPG cells. Treatment of a patient-derived DIPG cell line containing a de novo WIP1 mutation with the WIP1 inhibitor GSK2830371 resulted in significant growth inhibition. Combination treatment with GSK283031 and IR resulted in even greater inhibition of cell proliferation, compared to treatment with either modality alone. Our results suggest that WIP1 inhibition in combination with IR may provide a significant survival benefit in children being for DIPG.

108. WIP1 Modulates Responsiveness to Sonic Hedgehog Signaling in Neuronal Precursor Cells and Medulloblastoma

Wen, Jing; Nahta, Rita; Malhotra, Anshu; Brown, Briana D.; Kenney, Anna M.; Castellino, Robert

High-level amplification of the protein phosphatase PPM1D (WIP1) is present in a subset of medulloblastomas (MBs) that have an expression profile consistent with active Sonic Hedgehog (SHH) signaling. We found that WIP1 overexpression increased expression of Shh target genes and cell proliferation in response to Shh stimulation in NIH3T3 and cerebellar granule neuron precursor (cGNP) cells in a p53-independent manner. Thus, we developed a mouse in which WIP1 is expressed in the developing brain under control of the Neurod2 promoter (ND2:WIP1). The external granule layer in early post-natal ND2:WIP1 mice exhibited increased proliferation and expression of Shh downstream targets. MB incidence increased and survival decreased when ND2:WIP1 mice were crossed with a Shh-activated MB mouse model. Conversely, Wip1 knock out significantly suppressed MB formation in two independent mouse models of Shh-activated MB. Furthermore, Wip1 knock-down or treatment with a WIP1 inhibitor suppressed the effects of Shh stimulation and potentiated the growth inhibitory effects of SHH pathway-inhibiting drugs in Shh-activated MB cells in vitro. This suggests an important cross-talk between SHH and WIP1 pathways that accelerates tumorigenesis and supports WIP1 inhibition as a potential treatment strategy for MB.

109. Influence of Age on Bone Healing Following BMP-2 Delivery

Cheng, Albert; Krishnan, Laxminarayanan; Tran, Lisa; Williams, Joseph; Guldberg, Robert E.

Large bone defects remain one of the most challenging problems faced by orthopedic surgeons today. These defects are often the result of traumatic injury, congenital deformity, or tumor resection. Current treatments involve bone grafts and/or delivery of osteoinductive proteins such as bone morphogenetic protein 2 (BMP-2). But all BMPs are currently contraindicated by the FDA for use in pediatric patients due to the potential for massive inflammatory reactions, additional risks such as premature growth plate closure, and lack of appropriate dosing information. Despite this warning, off-label BMP-2 use still occurs in pediatric patients because there are currently no better alternatives. The objective of this study was to evaluate how age impacts bone healing, specifically the response to a clinically-relevant treatment such as BMP-2 delivery. We adapted our well-established critically-sized segmental bone defect model to

young (7-week-old) and old (8-month-old) rats, which is roughly the human equivalent of comparing a young adolescent to a middle-aged adult. Animals were treated with either 1 or 10μg BMP-2 delivered in a collagen sponge, the current clinical standard. We hypothesized that compared to older animals, young animals would be able heal at a lower BMP-2 dose but also exhibit increased inflammation at the higher BMP-2 dose. Early 1-week gene expression analysis of the regenerating bone defect tissue was performed targeting a panel of different genes. The young rats showed increased expression of genes linked to osteogenesis (RUNX2, COL1A1, OSX), chondrogenesis (SOX9, COL2A1, ACAN), and matrix remodeling (MMP2, MMP13). In contrast, the older rats had higher levels of inflammatory genes (IL1A, IL6, MCP1). Micro-computed tomography at 12 weeks revealed young rats had greater bone formation and higher bone mineral densities compared to older rats. Interestingly, only the young rats demonstrated significantly more bone when increasing the BMP-2 dose from 1 to 10μg. Finally, mechanical testing showed that the regenerated bones in young rats were stronger and had mechanical properties that were much closer to intact bone levels.To our knowledge, this is the first study to look directly at how age impacts the response to a bone healing treatment. We showed that there are early age-related differences in gene expression, and these result in long-term functional differences in bone regeneration. We also found that young rats are more sensitive to increases in BMP-2 dosing compared to older rats, suggesting a more conservative dosing strategy may be best for pediatric patients.

110. In Vitro Evaluation of Enhanced Respiratory Disease Immune Responses Associated with Formalin-Inactivated RSV Vaccine

Chirkova, Tatiana; Ha, Binh; Boyoglu-Barnum, Seyhan; Anderson, Larry J.

Respiratory syncytial virus (RSV) is a major cause of severe pneumonia and bronchiolitis in infants and young children and cause of repeated respiratory infections throughout life. Vaccine development against RSV is a high priority in pediatrics, yet no vaccine has been licensed. The first RSV vaccine, formalin-inactivated with alum adjuvant (FI-RSV), did not protect but instead was associated with enhanced respiratory disease (ERD) upon later RSV infection. In the clinical trial of FI-RSV vaccine, ERD affected young, presumably RSV naive, children but not older RSV primed children. We hypothesize that FI-RSV induced aberrant immunological memory in naïve children that with later RSV infection led to immune enhanced disease. Studying mechanisms underlying ERD will help to define aberrant immune responses induced by vaccination and could provide a pre-clinical system to determine ERD risk for candidate vaccines. Most pre-clinical studies use response to RSV challenge in vaccinated animals to evaluate ERD risk. We developed a more robust model focused on the aberrant memory response and not confounded by lack of virus replication and non-RSV antigens in challenge studies of vaccinated animals. We re-stimulate spleen cells of vaccinated mice with RSV peptides to directly assess the memory responses. In these studies, we show that FI-RSV vaccination compared to live virus infection induces higher Th2/Th17-bias in memory T cell responses. These responses were associated with production of IL-4 and IL-17 and intracellular expression of Gata-3 and ROR-γt after re-stimulation with RSV peptides. To evaluate ERD-associated immune responses in a human system, we studied responses to RSV and FI-RSV antigens in adult peripheral blood mononuclear cells (PBMC), i.e. responses from subjects with prior exposure to live RSV, and in cord blood mononuclear cells (CBMC), i.e. response from RSV naïve subjects. As an indicator of the memory development direction we assessed responses of dendritic cells (DC), and the phenotype they acquire after stimulation with FI-RSV or live RSV in vitro. FI-RSV, as opposed to live virus, induced predominantly Th2-directing phenotype in human DC with increased

expression of OX40L and decreased production of IL-12. This phenotype subsequently led to a development of Th2 responses in naïve CD4 T cells co-cultured with stimulated dendritic cells. These findings support a new approach for pre-clinical evaluation of a candidate RSV vaccine's ERD risk that should provide more reliable pre-clinical data from animal models and in vitro data on the likely human immune responses.

111. HIV-1 but not SIV Envelope Trafficking Requires the Endosomal Recycling Compartment and Rab11-FIP1C

Choi, Junghwa; Qi, Mingli; Ding, Lingmei; Wang, Jaang Jiun; Hammonds, Jason E.; Lapierre, Lynn A.; Goldenring, James R.; Spearman, Paul

We previously described an important role for Rab11-FIP1C (FIP1C) and Rab14 in mediating the incorporation of Env in a manner that depends upon the presence of an intact Env cytoplasmic tail (CT). FIP1C, also known as Rab coupling protein or RCP, is associated with membranes of the endosomal recycling compartment (ERC), where it plays an important role in the recycling of cargo to the plasma membrane. FIP1C/RCP includes a C-terminal Rab-binding domain that interacts with Rab 11, Rab 14, or Rab 4. We utilized a C-terminal FIP1C/RCP fragment (GFP-FIP1C560-649) as a dominant-negative molecule to disrupt Env trafficking through the ERC and as a tool to define motifs involved in FIP1C-dependent trafficking. GFP-FIP1C560-649 was predominantly localized in enlarged perinuclear endosomal structures, where it strongly colocalized with HIV-1 Env. Trapping of Env in the enlarged ERC was mapped to determinants in the cytoplasmic tail, and required both the membrane-proximal YXXL motif and a downstream motif previously identified as the putative FIP1C-binding domain. Using super-resolution microscopy and electron microscopy, the enlarged ERC was visualized as a series of interwoven tubular membranes in which Env and ERC markers are highly concentrated. We next examined the effect of GFP-FIP1C560-649 expression on SIVmac239 envelope incorporation. Remarkably, SIVmac239 Env evaded restriction by GFP-FIP1C560-649, and no trapping of SIV Env in the ERC was observed. This result suggested that the sequestering effect on Env by GFP-FIP1C560-649 is specific to the HIV-1 Env cytoplasmic tail. To further test this, we engineered an SIV-HIV Env chimeric molecule that contains the HIV-1 gp41 cytoplasmic tail and the SIVmac239 transmembrane segment and SU domain. GFP-FIP1C560-649 profoundly sequestered Env of the SIV-HIV Env chimera in the ERC, confirming the tail-specific inhibition of trafficking of GFP-FIP1C560-649. We conclude that the trafficking pathway utilized by HIV-1 Env utilizes ERC sorting and requires FIP1C, while SIV Env arrives at the particle assembly site via another route. These results support a model in which HIV-1 Env is directed to the ERC by endocytosis, followed by a FIP1C-dependent outward trafficking step that is required for particle incorporation.

112. Visual Engagement with Social Scenes as a Function of Physical Properties for School-Age Children with Autism Spectrum Disorder

Coben, Ella; Yurkovic, Julia; Stallworthy, Isabella; Jones, Warren; Klin, Ami; Shultz, Sarah

Background: While reduced engagement with the social world is a defining feature of Autism Spectrum Disorder (ASD), less is known about what is perceived as important to individuals with ASD. Building on previous research demonstrating that individuals with ASD show increased attention to physical cues,

including inanimate objects (Rice et al., 2012), objects in motion (Shultz et al. 2011), and audiovisual synchrony (Klin et al. 2009), the present study examines the extent to which viewers with ASD are engaged by physical cues within naturalistic social scenes. Viewer engagement is quantified by measuring patterns of eye-blink inhibition (Shultz et al., 2011). Probabilistically, people are least likely to blink when looking at what they perceive to be most important. Thus, by measuring change in rate of eye-blinking relative to ongoing scene content we can index engagement in viewers with ASD. Objective: Determine how physical properties within social scenes, such as motion, luminance, contrast, and saturation, influence engagement of children with ASD. Methods: Eye-tracking data were collected from 92 children with ASD and 44 age- and IQ-matched typically-developing (TD) children during viewing of movies depicting age-appropriate social scenes. Physical property values were quantified at each movie frame. Peristimulus time histograms were created to quantify percent change in blink rate relative to movie frames with physical property values exceeding a percentile threshold. A range of percentile thresholds were used to examine how engagement varies as a function of onscreen physical properties. Results: Preliminary analyses revealed that both TD and ASD viewers are highly engaged when viewing onscreen motion, with engagement increasing as onscreen motion increases. Both groups showed greater engagement immediately before and after motion events. However, level of engagement relative to motion events was significantly higher amongst ASD viewers even at lower motion thresholds. While motion modulates engagement for both groups, viewers with ASD showed a more pronounced increase in engagement that was also more closely time-locked with motion events compared to TD viewers. Conclusions: These findings suggest that viewers with ASD show greater sensitivity towards motion and perceive motion to be more salient compared to TD viewers. Future analyses will ascertain the influence of luminance, contrast, and saturation on visual engagement in viewers with ASD. These efforts will shed light on cues that engage individuals with ASD and may identify alternate viewing strategies used by these individuals to make sense of complex scenes.

113. Potentiators Exert Distinct Effects on Human, Murine, and Xenopus CFTR
Cui, Guiying

VX-770 (Kalydeco) has been approved for clinical usage in CF patients with several CFTR mutations. Yet the binding site(s) on CFTR for this compound and other small molecule potentiators are unknown. We hypothesize that insight into these questions could be gained by comparing the effect of potentiators on CFTR channels from different origins, e.g., human, mouse and Xenopus (frog). In the present study, we combined this comparative pharmacology approach with that of computer-aided drug discovery to identify and characterize new potentiators of CFTR. Our results demonstrate that: (1) VX-770 potentiated hCFTR, mCFTR, and xCFTR with different efficiencies; (2) NPPB and GlyH-101 both potentiated and blocked CFTR with different effects on hCFTR, mCFTR, and xCFTR; (3) P1 (VRT-532), P2 (PG-01), and P3 (SF-03) potentiated hCFTR under whole cell conditions; and (4) P1 and P2 potentiated WT- and D1152A-hCFTR in excised macropatches in a manner dependent upon the degree of PKA-mediated stimulation. High concentrations of P3 inhibited D1152A-hCFTR regardless of PKA concentration, while low concentrations of P3 potentiated D1152A-hCFTR under very low PKA stimulation. (5) P1 and P2 did not have additive effects, suggesting that these compounds might share binding sites; and (6) Using a pharmacophore modeling approach, we identified three new potentiators (IOWH-032, OSSK-2 and OSSK-3) that have structures similar to GlyH-101 and dual potentation / blocking activities on CFTR. These could potentially be developed into new compounds for the treatment of cystic fibrosis.

114. Designing Cancer Care for Kids, By Kids: Engaging Patients and Their Families in Creating More Efficient Patient-Centered Care

Denham, Megan E.; Busheri, Yousef; Sherrod, Amy; Lewis, Steven; Zimring, Craig; Wasilewski-Masker, Karen

Background: Children receiving chemotherapy at the Aflac Cancer & Blood Disorders Center (Aflac Cancer Center) at Children's Healthcare of Atlanta (CHOA) go through a complex process involving multiple steps, providers, and rooms. This complexity creates inefficiencies and unnecessary waits which consume time patients and families could spend doing what they want and need to do. Objectives: The global aim is to improve the experience of children receiving chemotherapy utilizing a patient-centered approach in which patients take an active role in describing, evaluating and improving the care they receive. Methods: Through an iterative process, the team developed a child-friendly process map for chemotherapy care steps and engaged patients, families, and clinicians to develop a "passport" data collection tool. Patients used the passport tool to collect data about their visit, including time stamps at the beginning and end of each activity and qualitative descriptions of feelings at each location in the process. A discrete event simulation model was then created using passport, arrival and staffing data. Results: Forty-eight oncology patients (ages 6 -16 years) documented their journeys with the passport tool. The mean visit duration was 169.7 minutes (range 45-438), and 49% of time was spent waiting (m= 82.7 minutes). Non-value added time as a percentage of total wait times included registration (5%), triage (9%), waiting for nurses (7%), and providers (9%), and for medications (45%). In the qualitative descriptions extended waits were frequently noted, particularly in the exam room. Negative emotions (scared, anxious or bored) were most commonly reported in the exam and infusion rooms where patients spent the most time waiting. Only positive emotions were reported in the "game" room (exam room waiting area). The infusion area also had more positive than negative emotions reported. Both of these areas have video games and other activities for patients. Conclusion: This research demonstrates a methodology to actively engage and empower pediatric oncology patients to improve the care they receive. The information will be used to identify and test opportunities to improve care by reducing the idle time spent during a chemotherapy visit by 20% in the next 6 months and improving the quality of idle time by targeting care points during which children are most unhappy or afraid. While waiting during visits is unavoidable, these results identify locations within the clinic to target improvements to optimize the patient experience.

115. Cancer Stem Cell Survival Post-Radiation in Medulloblastomas Requires YAP, YB1, and IGF2

Dey, Abhinav; Malhotra, Anshu; Kenney, Anna M.

Sonic hedgehog (Shh)-mediated medulloblastoma growth requires IGF2 (Insulin-like Growth Factor 2) and we have previously shown that Yes Associated Protein (YAP1) over-expression induces IGF2 expression as a part of YAP's radiation resistance program in mouse Shh-medulloblastoma cells and Shh-stimulated cerebellar granule neural precursors (CGNPs), proposed cells-of-origin for the SHH molecular subclass of medulloblastoma. We observe high levels of YAP and IGF2 in tumor cells occupying the peri-vascular niche, a microenvironmental niche proposed to house so-called tumor re-populating cells that survive radiation and contribute to medulloblastoma recurrence, which is fatal. The mechanism of IGF2 induction downstream of YAP is not understood. Using promoter binding-protein analysis, we have found that Y-box protein-1 (YB1) is a major regulator IGF2 expression downstream of

YAP and Sonic Hedgehog signaling, and YB1 also localizes to the peri-vascular niche. To better understand how YAP, YB1, and IGF2 regulate peri-vascular niche cell survival, we have developed an ex vivo approach using organotypic brain tumor slice cultures. Our data show that the population of perivascular niche cells expressing stem cell markers increases markedly following exposure to radiation, and that targeting any component of the YAP1-YB1-IGF2 axis increases the level of cell death within the niche and reduces niche expansion. These findings strongly suggest that therapeutic approaches designed to impair the function of this pathway could be used in combination with traditional tumor resection and chemotherapy, to reduce the use of cranio-spinal radiation of medulloblastoma patients, which causes life-long side effects that drastically impair quality of life. Future studies will include optimizing in vivo mouse models for studying radiation resistance and identifying additional functions for YB1 and YAP in tumor repopulating cells, both in their quiescent state and during radiation response.

116. Pediatric Migraine Treatment
diMonda, Richard

Our startup company, Sensory Innovations has licensed a neuromodulation platform from the Geffen School of Medicine at UCLA. We have developed a very simple, battery operated, non-invasive mechanical vibratory stimulator, which has been used to successfully treat chronic migraines and trigeminal neuropathy in a total of 20 pediatric and adult cases. The device applies a patent pending, specific vibrational waveform sequence to peripheral nerves located in the outer and inner auditory ear canal via a custom earplug applicator. Unique to this device is its apparent ability to significantly reduce pain within 45 minutes from the time of application, offering the potential to reduce the duration of a migraine event, which typically can last from between 4 and 72 hours. Ranking in the top 20 of the worlds most debilitating medical illnesses, migraines affect over 12% of the US population with an incidence of three times greater in women than men. Although it most commonly occurs in a person's peak productive years, between the ages of 25 and 55, migraines also affect a significant number of pediatric patients, ranging between 1.2 and 23%, depending upon age, with a wide range of disabling symptoms that often interferes with the ability to attend school, learn, and ultimately hold down a job. Pediatric migraines are more common in boys than in girls before puberty. As adolescence approaches the incidence and prevalence of migraine increase more rapidly in girls than in boys. At age 15, the prevalence of migraines is between 5% (boys) and 15% (girls). A prototype of the technology, suitable for human use, has been developed and is currently available for use in clinical feasibility trials, under an investigator initiated, hospital sponsored IRB. Office or hospital based research to explore a variety of pediatric headache conditions are being explored with physician and hospital sponsors across the southeast and in California.

117. Organotypic Slice Culture for Evaluating Treatment of Medulloblastoma
Felker, James; Dey, Abhinav; Metrock, Katie; Liu, Jingbo; MacDonald, Tobey; Kenney, Anna M.

Medulloblastoma (MB) is the most common malignant brain tumor of childhood. There are four 4 distinct molecular subgroups: Wingless (WNT), Sonic Hedgehog (SHH), Group 3, and Group 4. SHH comprise about 30% of all MB tumors and are among the most common in the very young and older children. Current treatments leave many of those who are cured with long term cognitive and/or physical

disabilities secondary to the treatment; thus, more effective alternative Shh-targeting strategies are needed. We developed a new assay, organotypic ex vivo slice culture (OSC), which retains the tumor microenvironment of SHH MB, and thus we hypothesize will be a robust predictive method for the evaluation of the efficacy and toxicity of a particular drug. We tested the STAT3 inhibitor (WP1066) that was used by the MacDonald lab against Medulloblastoma in vivo, as a single agent and in combination with radiation and other Shh pathway targeting agents, to 1) validate this assay and 2) to test for additional key molecular interactions in the Shh pathway that may be amenable for future targeting. Methods:For generation of slice cultures, 300 mm sagittal sections of murine SmoA1 MB were generated using a vibratome. The slices were placed on trans-well inserts on a 6-well plate. Slices are cultured in appropriate media for 24 hours, than irradiated, exposed to Vehicle or various concentrations of Shh pathway inhibitor (LDE225) and/or STAT3 inhibitor (WP1066), and grown for an additional 24 hours. The sample was fixed and immunostained with antibodies against markers of cell death (cleaved caspase-3 (CC3)), as well as stem cell marker (CD15), followed by secondary fluorescent-labelled antibodies. The samples were then imaged by confocal microscopy. Quantification of staining was done with ImageJ software. Results were compared to the in vivo results by the MacDonald lab to determine the correlation of the ex vivo assay with in vivo data. Results/Conclusion:In murine SmoA1/Math1 MB tumor slice cultures, we observed a dose-dependent increase in CC3 with WP1066. This efficacy correlated with in vivo data showing decreased rate of change in tumor volume in mice treated with WP1066. We found minimal cell death in normal brain tissue. We also found that WP1066 decreased growth of CD15-positive neural stem cells after radiation treatment. In conclusion, ex vivo slice culture of brain tumors shows efficacy as a predictive method of evaluation of drug, as well as evaluation of the tumor microenvironment.

118. A Systematic Review of Transitional Care for Adolescents and Emerging Adults with Diabetes
Findley, M.K.; Cha, EunSeok; Wong, Eugene; Faulkner, Melissa

Background: The prevalence of diabetes and prediabetes in adolescents and emerging adults is increasing, thus also expanding the transition from parent-directed pediatric to self-managed adult healthcare; however, there is currently no consensus model for this transition in the United States. A systematic review was completed in order to examine the level of evidence from primary research for the process and outcomes of transitional care programs for adolescents and emerging adults with either type 1 diabetes, type 2 diabetes, or prediabetes. The intent of this review was to provide a synthesis of the best evidence to inform clinicians, researchers, and policy makers of key components of a model of high quality transitional care. Methods: The systematic review was conducted following the Joanna Briggs Institute guidelines and the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement. The initial literature search identified 772 articles related to health care transition, diabetes, adolescents and emerging adults published between 2004 and 2014. Exclusion criteria included non-research articles and non-full text articles. Duplicates were removed and articles with titles, abstracts, or full text that did not fit within the exclusion criteria were also removed, leaving a total of 31 articles for the synthesis of information included in this review. Results: Studies focused on those with type 1 diabetes, not type 2 diabetes or prediabetes, thus identifying a major gap in the existing literature. Studies were primarily descriptive in nature and many were conducted outside the United States. Major findings and conclusions include differences in pediatric versus adult care delivery, factors effecting pediatric transition preparation, the importance of structured transitional programs, and suggestions for

successful health care transition including enhanced communication between pediatric and adult care providers, age-specific clinics, and adult providers tailoring information for emerging adults' concerns. Findings from the extant research have been incorporated into position statements of United States organizations on transitional care without further testing of the currently available interventions. Implications include future research on program development, implementation, and evaluation that is inclusive of adolescents and emerging adults, regardless of diabetes type, or prediabetes. In order to develop a consensus model for a seamless system of transitional care, quality research and incorporating perspectives of patients, parents and providers must be conducted and evaluated systematically to enhance diabetes management, quality of life, and prevention of long-term complications.

119. The Clinical Utility of Exercise Stress Echocardiography: How Does it Impact Clinical Decision Making in Pediatric Patients?

Friedman, Heather; Allen, Nicole; Stark, Megan; Sachdeva, Ritu; Border, William

Background: Exercise stress echocardiography (ESE) is one of the cornerstones of non-invasive assessment of ischemia in adults. However, its role in the pediatric population is less well defined. It takes a considerable commitment to resource such a program in terms of space, staffing, training, technology and equipment. We sought to determine its utility and impact on clinical decision making in a large pediatric cardiac center. Methods: Records of all patients who underwent an ESE at our center from 2011 to 2015 were reviewed for patient demographics, indications for ESE, and test results. Test results were categorized into normal or abnormal. The potential outcomes of the test were categorized into: continue current clinical management; unrestricted from exercise; restricted from exercise, which was further subcategorized into restricted only from varsity or competitive athletics, restricted from recreational athletics, and restricted from all exercise including physical education; medication change; and surgical referral. A change in clinical decision making was evaluated by reviewing pre- and post-test decision making by the ordering cardiologist. Results: During the study period, 381 ESE's were performed (mean age 13.9 ± 0.17 years, 262 (68.8% males). The indications included: congenital heart disease in 106 (28%) (tetralogy of Fallot in 15, transposition of great arteries in 24, single ventricle in 12, and other in 49); hypertrophic cardiomyopathy (HCM) in 77 (20%); chest pain or syncope in 89 (23%); coronary anomalies in 41 (11%); left ventricular hypertrophy in 17 (4.5%); and other indications in 51 (13%). Of all the tests, 281 were normal, and 100 were abnormal. Clinical management changed as a result of ESE in 168 (44.1%). Of those that changed clinical management, 118 (70.2%) were cleared for exercise, 39 (23.2%) were restricted from exercise in some way, 3 (1.8%) had a change in their medication, and 8 (4.8%) were referred for a surgical intervention. In patients with HCM, the most common single lesion indication, clinical management was changed in 42 (55%). Conclusion: ESE provides the pediatric cardiologist with useful information that impacts management in a wide variety of pediatric cardiac disorders. Clinical management changed in nearly half the patients that were subjected to an ESE at our center. This supports the value of ESE for informing clinical decision making. Future studies should further refine patient selection and examine its impact on patient outcomes.

120. Anti-Microbial Modifications of Medical Tubing to Reduce Bacterial Adhesion
Geoghan, Allison; Beveridge, Jennifer; Finn, M.G.

Numerous conditions, including ventilator-associated pneumonia and catheter-related urinary tract infections, are linked to the formation of biofilms and colonization of bacteria on medical tubing, which then leads to a bacterial infection. These conditions are associated with high mortality rates, as well as increased hospital stay lengths and medical care costs. Currently, using silver-coated tubing is the main effort to minimize these infections in intubated patients, however, this is not FDA approved for pediatric use. To address this problem, we propose a covalent linkage of known anti-microbial compounds to existing medical tubing to reduce/eliminate the growth of pathogenic bacteria on this material. Using medical tubing, Covidien Mallinckrodt cuffless endotracheal tubing (4.00 mm ID), we first modify the surface with an azide functional group, followed by covalent attachment of anti-microbial agent via the Cu-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. These materials will then be tested for bacterial adhesion using hospital-relevant strains: P. aeruginosa, S. aureus, E. coli. This method will allow us to screen a variety of different modifications in a time-efficient manner to develop an alternative strategy to minimizing biofilm and colonization of bacteria on medical tubing.

121. Dual Mode Signal Amplification with Hemin Nanoparticles for Detection of Disease
Gibson, Lauren; Wright, David

The developing world suffers dramatically from child mortality. In these areas, there is a pressing need for sensitive and stable assays for disease diagnosis that require minimal electricity and expertise. In the developed world, enzyme-linked immunosorbent assays (ELISAs), are prolifically used for disease diagnosis, but the horseradish peroxidase enzyme is expensive and sensitive to light and other environmental conditions. Since the infrastructure required to perform these assays is not available in low-resource areas, new strategies for disease detection that are stable, cost-effective and sensitive are vital. In this work, a new detection strategy has been developed based upon hemin nanoparticles. The use of these nanoparticles eliminates the need for a protein enzyme, greatly increasing the stability of the assay. The hemin nanoparticle detection system employs a dual amplification strategy. The first type of amplification achieved is nanoparticle-based amplification. This occurs when a hemin nanoparticle, which is bound to a biomarker of interest, is broken apart by pyridine into individual hemin molecules. Thus, instead of one nanoparticle, there are thousands of signal producing hemin molecules. The second form of amplification occurs through the catalytic turnover of a substrate, by hemin, in the presence of hydrogen peroxide. This dual amplification strategy produces a sensitive assay, resulting in low picomolar limits of detection for the model biomarker, IgG. Thus, the hemin nanoparticle detection method is sensitive as well as stable, a combination of characteristics not commonly found in ELISAs. As the hemin nanoparticle method is a platform technology, it can be applied to the detection of many diseases. Currently, the assay is being optimized for the detection of the malarial biomarker Plasmodium lactate dehydrogenase (pLDH).

122. Correction of Diamond-Blackfan Induced Pluripotent Stem Cells via CRISPR

Westin, Erik; Devadasan, Divya; Li, Chao; Ding, Lei; Townes, Tim; Goldman, Frederick

Diamond Blackfan Anemia (DBA) is a congenital bone marrow failure disorder characterized by severe erythroblastopenia. Impairment of red blood cell production arises due to mutations in ribosomal genes that lead to a deficiency in ribosomal protein synthesis. Standard treatment for DBA patients include steroids or red blood cell transfusions, while at this time the only curative option that exists is an allogeneic hematopoietic progenitor cell (HPC) transplant. Induced pluripotent stem cells (iPSCs) provide a means to manufacture HPCs from one's own cells and could expand the utility of gene correction strategies in disorders of hematopoiesis. We hypothesized that acquisition of DBA fibroblasts for the creation of iPSCs could provide an in vitro model recapitulating defects in erythroid development. In addition, correction of this mutation might alleviate the presumed block in erythropoiesis. To this end, we generated five iPS cell lines (using an integration-free, novel six-factor episomal construct) from a DBA patient carrying a non-sense RPS19 mutation (R94X). Experiments characterizing iPSCs ability to generate HPCs (via OP9 mouse stromal cell co-culture) and undergo erythropoiesis demonstrate an 8-9 fold decrease in production of both CD71+/CD235+ and CD71-/CD235+ in DBA cells compared to controls. In order to correct mutated iPSCs, CRISPR targets were evaluated and optimized in 293T HEK cells and DBA fibroblasts. This approach has led to optimization of an allele-specific targeting complex. Continued attempts to improve correction efficiency of DBA iPSCs have included protocols adopting in-house manufacturing of the Cas9 protein component. This protein is complexed with in vitro transcribed guide RNA to produce a double-stranded cut and subsequent homologous recombination with a 60mer single-strand oligo in cells. Using this strategy, coupled with droplet digital PCR, we have attained a correction efficiency of ~40% that permits recovery of corrected cells via single-cell cloning. Importantly, corrected DBA cells are more proficient in their ability to generate CD34+ progenitors and erythrocytes compared to uncorrected counterparts. Additional studies are underway utilizing CRISPR correction strategies in other cells of hematopoietic origin.

123. Optimizing A Non-Ablative Conditioning Regimen For Hematopoietic Stem Cell Transplantation In A Murine Model Of Sickle Cell Disease

Devadasan, Divya; Sun, Chiao-Wang; Townes, Tim; Goldman, Frederick

Background: Hematopoietic stem cell transplantation (HSCT) for patients with sickle cell disease (SCD) is curative, though significant toxicity from myeloablative conditioning has limited its utility. Reduced intensity conditioning (RIC) regimens have been used for several malignant and non-malignant disorders, including SCD, to decrease transplant toxicity. However, RIC regimens are associated with higher rates of incomplete donor chimerism/rejection. Strategies to improve engraftment with RIC regimens are under development, including use of aklyating agents with lower toxicity profiles (Treosulfan) and c-kit modifers (ACK2). We have previously developed knock-in mice producing sickle human hemoglobin which recapitulates severe anemia, hyposthanuria and limited lifespan found in SCD patients. Similarly, these animals also have a poor tolerance to radiation conditioning. Objective: To optimize RIC in a murine model of SCD that allows for sufficient donor RBC chimerism and disease amelioration. Design/Methods: Mice received varying conditioning regimens (+/- rescue with control marrow), including irradiation (TBI), Treosulfan (2-5g/kg; IP), or ACK2 (100-500ug; IP). The hematologic effects of respective treatments were determined by assessment of marrow cellularity, peripheral CBCs, and RBC donor chimerism (by Iso-

electric focusing). Urine concentrating ability, a surrogate of renal damage, was also assessed. Results: Erythroid hyperplasia was noted in the BM of SCD mice. ACK2 and Treosulfan, independently and in a dose dependent manner, decreased bone marrow cellularity and induced pancytopenia in control and SCD mice. Overall, the maximum tolerated dose of Treosulfan, ACK2 and TBI was ~50% lower in SCD mice compared to controls. While Treosulfan-treated SCD mice (3g/kg) had donor cell engraftment at 2 weeks post-BM rescue, only 4/16 sustained long-term complete donor RBC chimerism. In contrast, all control mice (humanized hemoglobin beta A) treated with a non-ablative dose of Treosulfan had evidence of sickle RBC chimerism. Combinatorial use of ACK2 and Treosulfan did not enhance engraftment rates compared to Treosulfan alone. Normalization of reticulocyte counts and urine osmolality was found in SCD mice with sustained donor RBC chimerism while non-engrafted mice remained hyposthanuric. Importantly, fertility was preserved in transplanted SCD animals with complete donor chimerism. Conclusion: SCD mice closely mimic human disease in phenotype and ablative conditioning intolerance. Non-ablative dosing of Treosulfan permitted engraftment in control animals, though in only a subset of SCD-transplanted animals. Possible explanations for incomplete engraftment in SCD mice include immune mediated rejection or pre-existent marrow hypercellularity. Future experiments in SCD animals will include agents to reduce marrow cellularity pre HSCT and inhibit recipient immune responses.

124. 3-D Spheroid Pacing Unit by Conversion of Cardiomyocytes to Pacemaker Cells
Gonzalez, Sandra; Sung, Jung Hoon; Cho, Hee Cheol

Children who suffer from congenital and rhythm disorders require a pacemaker device from birth, with most remaining dependent for their lifetime. Even though these devices pose a solution, they require invasive surgeries for battery replacements as well as new leads as the child grows. As a result, there has been a shift in the research field to develop biological pacemakers which consist solely of the child's own cells. We have previously generated pacemaker cells from stem cells or through gene therapy by reprogramming ordinary cardiac muscle cells. Previously we have transformed ordinary cardiac myocytes to pacemaker cells with a transient expression of a single transcription factor, Tbx18. The de novo pacemaker cells, called induced SAN-like pacemaker cells (iSANs), display all hallmarks of native, freshly-isolated pacemaker cells in their morphology and electrophysiology. This method provides a unique opportunity to study pacemaker cell biology, being able to attain hundreds of iSANs from each round of reprogramming. Here, we propose to engineer a 3-dimensional pacemaker tissue to recapitulate the native pacemaker tissue, the sinoatrial node (SAN). In order to create this 3D model of the SAN we generated spheroids using Aggrewell plates. Our spheroids consisted of 1000 freshly isolated neonatal rat ventricular myocytes reprogrammed with adenovirus CMV-GFP or CMV-TBX18-IRES-ZsGreen. We allowed the spheres to mature in suspension cell culture for one to two weeks post gene delivery. The spheres were placed on multi-electrode arrays, which enables measurements of beating rate and conduction velocity over three weeks post adenovirus delivery. In addition quantitative PCR was performed for detection of gap junction genes Cx43, and Cx45. Our results show that Tbx18 spheres were able to maintain a beating rate of 100 beats per minute for >three weeks, whereas a monolayer of Tbx18 cells were viable for <two weeks. In addition, the conduction velocity of the spheres decreased over time, more closely mimicking the native SAN. Lastly, the qPCR data demonstrated an increase in Cx45 while there was a decrease of Cx43 in the Tbx18 group. In conclusion, the 3D Tbx18 spheroid pacing units closely replicated the salient features of the innate SAN tissue. The spheres were able to maintain autonomous and spontaneous action potentials as well as replicating the transcriptional pattern

seen in the SAN. The spontaneously-pacing spheroids provide a platform for designing engineered SANs, which could be implanted in children who suffer from rhythm disorders.

125. Immunogenicity of VLP-Based RSV G peptides
Ha, Binh; Jadhao, Samadhan; Chen, Xuemin; Spearman, Paul, Anderson, Larry J.

Respiratory syncytial virus (RSV) is the single most important cause of serious lower respiratory tract infections in young children and infants worldwide. Symptoms of RSV infections include fever, bronchiolitis, bronchitis, and pneumonia. It is estimated that RSV causes 64 million infections each year globally, from which 60,000 to 220,000 are resulted deaths. The heavily glycosylated surface proteins G and F mediate viral attaching and fusion, respectively, and have been shown to induce protective immune responses but doing so ineffectively. We hypothesized that vaccination using virus-like particles (VLPs) platform would be immunogenic and a safe approach to study RSV vaccines. We designed different G constructs containing the conserved motif CX3C and used these as VLPs-based antigens in a mouse immunization model. Preliminary data show that all but FL CX4C are immunogenic, suggesting that VLPs with G antigens are promising to study RSV vaccines.

126. Vitamin D Supplementation Decreases Immune Activation and Exhaustion in HIV+ Youth
Habib, Jakob; Eckard, Allison; Chahroudi, Ann; McComsey, Grace; Rosebush, Julia; Daniels, Julie; Uribe-Leitz, Monika; Tangpricha, Vin

Background: Heightened immune activation and exhaustion drive HIV disease progression and co-morbidities. Vitamin D has pleiotropic effects on the immune system, but little is known about the effects of supplementation in HIV infection. Our study investigates these potential effects after 12 months of supplementation in virologically-suppressed HIV+ youth with vitamin D insufficiency. Methods: This is a randomized, active-control, double-blind trial investigating two different monthly vitamin D3 doses [60,000 (medium) or 120,000 (high) IU/month] vs. a control arm of 18,000 IU/month in 8-26 year old HIV+ youth on ART with baseline 25-hydroxyvitamin D (25(OH)D) ≤30 ng/mL and HIV-1 RNA <1000 copies/mL. Randomization was stratified by EFV use. Only subjects who maintained an undetectable HIV-1 RNA level for 12 months and had available PBMCs at baseline (BL) and 12 months were included in this analysis. Markers of immune activation and exhaustion were measured by flow cytometry. Comparisons of marker changes from BL to 12 months were made within each of the three dosing groups and between groups (medium vs. control, high vs. control) using appropriate two-sample tests. Results: 50% of enrolled participants (N=51) were included in the analysis: 63% male, 86% black with median (Q1, Q3) age of 20 (15, 22) years and CD4 count of 654 (451, 888) cells/mm3 (all similar to the rest of the study participants). HIV and ARV duration were 11 (3, 18) and 7 (2, 11) years, respectively. Overall, BL 25(OH)D was 17 (13, 25) ng/mL and not different between arms or to the rest of the study participants. By 12 months, 25(OH)D increased statistically within each dosing group (control: +12 (6, 17); medium: +19 (10, 32); high: +31 (16, 41) ng/mL; all P<0.001) with greater increases in the medium group vs. controls (P=0.04) and high group vs. controls (P=0.008). Overall, all measured markers decreased with CD4 activation (CD4+CD38+HLA-DR+), CD8 activation (CD8+CD38+HLA-DR+), CD4 exhaustion (CD4+CD38+HLA-DR+PD1+), and inflammatory monocytes (CD14+CD16+) reaching statistical significance. These decreases were mostly driven by subjects in the high dose group. Conclusions:

Vitamin D supplementation decreased markers of T-cell activation and exhaustion, and monocyte activation regardless of dose in HIV+ youth, but subjects given the highest dose (120,000 IU/month) showed the greatest improvements. These data suggest that high-dose vitamin D may further attenuate immune activation and exhaustion HIV+ youth on ART.

127. Background Phase Correction in Congenital Heart Disease: Does Reliability Vary Based on Underlying Disease Type?

Hashemi, Sassan; Ramamurthy, Senthil; Parks, W. James; Sallee III, Denver; Slesnick, Tim

Background: Phase-contrast magnetic resonance (PC-MR) allows non-invasive calculation of vascular flow, peak velocities and shunts. The technique, however, has inherent limitations, one of which is background phase errors. Various background phase correction (BPC) algorithms have been developed. The aim of this study is to apply various commercially available BPC algorithms in pediatric patients with a variety of disease types. Methods: Retrospectively, we analyzed patients in 4 categories: normal anatomy, stenotic aortic valve, dilated aortic root, and regurgitant pulmonary valve. All patients had PC-MR data obtained on a 1.5T magnet (Siemens MAGNETOM Aera) using a product sequence optimized for pediatric patients with free breathing techniques, multiple signal averages, and the vessel of interest placed at isocenter. We excluded patients with intracardiac shunting, arrhythmias or prosthetic valves. We calculated the aortic to pulmonary flow ratio (Qp:Qs) on 5 different analysis platforms both with and without BPC. Three platforms utilize a full-field stationary tissue fit and two others use a region of interest (ROI) for BPC, which was placed in stationary tissue as close to the vessel of interest as possible. One expert reader performed all vessel segmentations, ensuring all segmentations on different platforms used similar technique. Qp:Qs between 0.9 – 1.1 was defined as clinically acceptable. Results: Fifty patients (76% males, mean age=13±5 years) were analyzed (20 normal, 10 each from the other groups). The intraclass correlation coefficient for intra-observer reliability was 0.99. Distributions of Qp:Qs for different disease categories, before and after BPC, on all platforms are summarized in Table 1 and Figure 1. Non-corrected (NC) Qp:Qs in normal patients are distributed in the clinically acceptable range. The worst underestimation of Qp:Qs occurred in the regurgitant group, and BPC was significantly beneficial for these patients, with platform 5 being the most efficient. In the dilated aorta group, only the outlier Qp:Qs measurements benefitted from BPC, but the distribution of data were mostly in the acceptable range. BPC could not compensate for outlier measurements in the stenotic aortic valve group, though the medians of distribution did not deviate from 1.0. Conclusion: Non-corrected phase contrast values vary in clinical accuracy based on underlying disease type, and there are significant differences between various vendors' BPC algorithm efficiencies at resolving these discrepancies. These effects were most pronounced in pediatric patients with regurgitant lesions.

128. Stromal Cell Gas6 Protects Leukemia Cells from MERTK Inhibition

Huey, Madeline; Hill, Amanda; Minson, Katherine; Wang, Xiaodong; Frye, Stephen; Earp, Shelton; DeRyckere, Deborah; Graham, Douglas

Background: Inhibition of MERTK with the small molecule inhibitor MRX-2843 decreases tumor burden and prolongs survival in acute leukemia cell line and patient-derived xenograft models. However, while treatment with MRX-2843 reduces leukemia in peripheral blood and spleen, it is less effective in clearing

the bone marrow of leukemic blasts. Gas6, a MERTK, Axl, and Tyro3 ligand, is a poor prognostic factor in AML, mediates increased resistance to cytotoxic chemotherapy in leukemia cells, and is present in the bone marrow stroma. The objective of this work was to determine if bone marrow stromal cell Gas6 induces resistance to MRX-2843. Design/Method: Acute leukemia cell lines were cultured in the presence of Gas6-producing fibroblast-like cell lines or bone marrow derived stromal cells (BMDSCs) from wild type or Gas6 knockout mice. Axl-FC was added to co-cultures to bind and deplete Gas6. Induction of apoptosis and cell death was determined by flow cytometry after treatment with MRX-2843 or vehicle. Expression of MERTK and related kinases AXL and TYRO-3 was determined by immunoblot. Results: Co-culture with Gas6-expressing cell lines significantly reduced leukemia cell death in response to treatment with 300 nM MRX-2843 compared to leukemia cells alone (18.25% versus 93.09%, p<0.001). The protective effect was dose-dependent and reduced by titration of Gas6 with 1 ug/mL Axl-FC (7.4% versus 16.5%, p=0.022). Similarly, BMDSCs from wild type mice protected leukemia cells from MRX-2843 induced cell death more effectively than BMDSCs from Gas6 knockout mice (4.2% versus 72.39%). Immunoblot analysis demonstrated increased expression of TYRO-3 in leukemia cells in response to co-culture. Conclusions: The MERTK ligand Gas6 is produced by fibroblast-like cells lines and BMDSCs and mediates protection of leukemia cells from cell death induced by MERTK inhibition with MRX-2843. Gas6 depletion using genetic (GAS6 knock out mice) and biology (Axl-FC) strategies decreased resistance of leukemia cells to MRX-2843 in the presence of stroma. These data are consistent with a mechanism by which increased expression and activation of TYRO-3 and/or AXL promotes cell survival and resistance to selective MERTK inhibition. Further, combined treatment with MRX-2843 and a bone marrow mobilizing agent may be an effective therapeutic strategy.

129. Development of EIA Assays to Evaluate Antibody Response to RSV Infections in Children
Jadhao, Samadhan; Ha, Binh; Chirkova, Tatiana; Rosas-Salazar, Christian; Hartert, Tina; Anderson, Larry J.

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory virus infections in infants, immunocompromised individuals and the elderly. Despite many decades of research and development, licensed RSV vaccines are not available. Making availability of RSV antibody assays to detect and quantify antibodies to RSV infection is important to not only assessing response to a vaccine but also for detecting past infection and assessing disease severity and burden and primed versus naÃ¯ve status to accurately assess a vaccines impact on disease. We developed EIA using antigens from strains representing the two major antigenic and genetic RSV groups, A and B, and G protein central conserved region synthetic peptides as antigens. We screened plasma specimens for RSV antibodies from 100 known RSV infected children. These EIA assays reliably detect antibodies in known RSV-infected children.

130. Downregulation of a G Protein-Coupled Receptor Inhibits Cardiomyocyte Differentiation while Potentiates Endothelial Cell Differentiation from Human Pluripotent Stem Cells
Jha, Rajneesh; Singh, Monalisa; Wu, Qingling; Preininger, Marcela K.; Xu, Chunhui

Differentiation of human pluripotent stem cells (hPSCs) to cardiomyocytes and endothelial cells is tightly regulated by signaling molecules and transcription factors. Hence, understanding molecules involved in

their lineage commitment and differentiation is important for exploring their potential use for cell therapy and drug testing. Here, we have identified a G Protein-coupled receptor (GPCR) that is transiently upregulated during early stages of growth factor-mediated cardiomyocyte differentiation from hPSCs. Knockdown of the GPCR using short hairpin RNA (shRNA) did not affect undifferentiated cell morphology, growth and expression of pluripotency markers, but reduced the expression of mesodermal and endodermal lineage marker expression upon the growth factor induction. Moreover, knockdown of the GPCR also significantly reduced the expression of key cardiac transcription factors and other cardiomyocyte-associated genes, leading to poor differentiation. Interestingly, knockdown of the GPCR directed mesoderm towards endothelial cells as shown by upregulated expression of endothelial cell markers. Differentiation of endothelial cells was further confirmed by in vitro functional assays including their ability to form capillary-like structures and uptake acetylated low density lipoproteins. These data suggest that the GPCR is an important molecule for both cardiac and endothelial cell lineage commitment.

131. A Retrospective Investigation of the Effectiveness of the Providence Orthosis for Scoliosis
Johnson, Marjorie; Davis, Catherine; Murphy, Joshua; Schmitz, Michael; Flanagan, Jill; Dennis, Devito; Cash, Kaitlin; Stanislaus, Mellany

Introduction: The Providence orthosis is a back brace worn at night with the goal of preventing progression of scoliosis curvature in skeletally immature patients. Previous studies have reported widely varying success rates. Methods: Seventy four subjects who were treated with Providence orthoses for idiopathic scoliosis were retrospectively identified. Subjects had initial curve magnitudes of 20-45 degrees, were four years old or older, were Risser 0, 1, or 2 and, if female, were premenarcheal or <1 year post menarcheal. Cobb angles were measured and compared on their initial x-rays and latest available x-rays. Time between initial and follow up x-rays ranged from 365 days to 1351 days, with a mean of 592 days (1.6 years). Results: Overall, 61% of subjects did not experience >5 degrees of curve progression and 89% of subjects did not progress to surgical level (50 degrees). With success defined as ≤5 degrees of curve progression, more inferior curve apex, lower Cobb angle, and increased age were all found to be correlated with higher success rates. Single lumbar and thoracolumbar curves had the highest success rates at 75% and 65% respectively. For double major curves, the success rate dropped to 52%. For curves ≥ 35 degrees, the success rate was only 33%. Conclusion: The Providence orthosis can be effective at changing the natural history of idiopathic scoliosis. It is most likely to be successful with single lumbar and thoracolumbar curves and with curves ≤35 degrees.

132. Association of Pre-Operative Inflammatory Markers with Post-Operative Outcomes Following Repair of Congenital Heart Disease
Jones, Shannon; McCracken, Courtney; Mahle, William; Oster, Matthew E.

Background:While there are some well-established predictors of mortality in patients undergoing repair of congenital heart disease (CHD), much remains unknown regarding differential outcomes following surgical intervention. Inflammatory markers have been shown to have important associations with outcomes in adult cardiac patients, but research evaluating such associations in pediatric patients is lacking. Our objective was to determine whether pre-operative inflammatory markers, including lymphopenia, thrombocytopenia, and neutrophilia, were associated with increased morbidity and mortality

following cardiac surgery. Methods: We performed a retrospective cohort study on children (<19 yo) who underwent cardiac surgical repair between 2004 and 2014 at our institution. Data collected included pre-operative cell counts from most recent complete blood count obtained within seven days pre-operatively, age at surgery, gender, self-reported race/ ethnicity, presence of a genetic syndrome, STS-EACTS Congenital Heart Surgery Mortality Category (STAT category), mortality status, and post-operative length of stay. We performed multivariable logistic regression to determine the association of lymphopenia (lymphocyte count ≤ 3000 cells/μL), thrombocytopenia (platelet count < 150,000/ μL), and neutrophilia (neutrophil count ≥ 7000 cells/ μL) with in-hospital mortality and performed multivariable linear regression to determine the association of cell counts with post-operative length of stay. Results: Of the 4865 patients, 28% were aged <1 month, 36% 1 month-1 year, and 37% >1 year. Patients were predominantly white (48%), male (54%), did not have a genetic syndrome (82%), and underwent category STAT 1 surgery (41%). Overall mortality was 6%, and median length of stay was 6 days. Lymphopenia and neutrophilia were associated with increased post-operative mortality, but thrombocytopenia was not. Each of the inflammatory markers was associated with longer adjusted length of stay: approximately one day for lymphopenia, three days for thrombocytopenia, and two days for neutrophilia. Conclusions: Pre-operative lymphopenia and neutrophilia are associated with increased risk of both post-operative mortality and longer length of stay in patients undergoing surgical repair of CHD. Pre-operative thrombocytopenia is associated with only longer length of stay post-operatively. Further research should be carried out to determine if pre-operative intervention on any of these findings would result in improved mortality or shortened post-operative length of stay. In the interim, knowledge of a child's cell counts pre-operatively may hold important prognostic value during pre-operative surgical planning.

133. Subtype-Specific Cellular Composition of the Glioblastoma Microenvironment

Kaffes, Ioannis; Szulzewsky, Frank; Alikhanyan, Kristina; Brat, Daniel J.; Hambardzumyan, Dolores

Glioblastoma (GBM), a grade IV astrocytoma, is the most common malignant primary brain tumor. With a 5-year survival rate of less than 10% and a median survival of 15 months it remains a largely incurable disease. Efforts have failed to create novel treatment options conferring an improved prognosis for patients. Even though the disease is most common in the sixth through eighth decennium, glioblastoma can, in rare cases, also affect children, with a similarly dismal prognosis. Our current understanding of the complex biology of gliomas is largely derived from studies of genetic and molecular changes within cancer cells. Specifically, recent characterization of the genome, epigenome and transcriptome of GBM has provided a higher-resolution picture of their alterations, revealing four different subtypes (classical, mesenchymal, proneural and neural) with distinct molecular signatures. Utilizing gene expression data from The Cancer Genome Atlas Project and the Gene Expression Omnibus database, several studies have demonstrated an enrichment of immune response-related gene expression, especially microglia/macrophage genes in the mesenchymal subtype of glioblastoma compared to the other subtypes, suggesting that there may be differences in the cellular composition of those distinct groups. In order to investigate the differences in the cellular composition of the tumor microenvironment, we used immunohistochemical analysis of various human GBM subtypes. Different cell-specific markers were used to define distinct cell types: to characterize immune infiltration, we used Iba1 as a marker for tumor associated macrophages, FOXP3, representing regulatory T cells, as well as CD4 and CD8, which are mainly expressed by T helper and cytotoxic T cells, respectively. Other cells constituting a relevant part of the tumor microenvironment, including endothelial cells and pericytes, were visualized using CD31 and

Smooth Muscle Actin. NeuN was used to identify neurons. Finally, cell proliferation was analyzed using Ki67. A clearer picture regarding the subtype-specific cellular context in glioblastoma is necessary in order to create an improved understanding of the role of the tumor microenvironment in GBM pathogenesis and to potentially design personalized treatment options in the future.

134. Electromechanical Characterization of an Implantable Sensor-Based Early Warning System for Non-Union in Pediatric Bone Fusion

Klosterhoff, Brett S.; Tsang, Melissa X.; Ong, Keat Ghee; Allen, Mark G.; Guldberg, Robert E.; Willett, Nick J.

Background: For pediatric patients, it is difficult to monitor the success of a bone fusion procedure. Computed tomography is the current clinical standard to assess bone fusion. However, it is imperative that radiation exposure is curtailed in pediatric populations. Thus, radiographic imaging is usually minimized and complications in bone fusion are challenging to diagnose until evident failure has occurred. There is a clear clinical need for a minimally invasive metric that will enable clinicians to longitudinally monitor the progression of a bone fusion procedure. The objective of this work was to develop an implantable sensor that wirelessly transmits real-time measurements of fusion success in vivo. Methods: To monitor the success of bone fusion, we have fabricated sensors which mount to an internal fixation plate and measure the mechanical deformation across the defect interface in our rodent segmental defect model. The sensors developed for this application utilize evaporated titanium strain-gage elements and gold connection lines patterned on a flexible polyimide substrate. The sensors were encapsulated in a conformal parylene C coating to provide biocompatibility and passivation. Ex vivo mechanical testing of sensor-instrumented fixation plates was performed in axial and off-axis compression under physiologically relevant cyclic loads to evaluate the strain sensing capabilities of the sensors. For off-axis testing, the defect space was filled with surrogate materials of varying stiffness to simulate plate strains at different time-points in healing (empty - initial injury; polyurethane rubber - unmineralized soft tissue callus; teflon - woven bone). Results: Axial ex vivo mechanical testing results established that the custom-fabricated strain sensors were functional and linear over the physiologically relevant strain range. Additionally, off-axis testing validated that sensors were able to detect and differentiate bridged defects from unbridged defects, indicating the devices could be used as a bone fusion sensor. Analog circuitry components for wireless data transmission have been identified to ensure safe operation of the sensor for 12 weeks in vivo with sufficient battery power to acquire 14 days of continuous strain data. This will be further extended by upcoming work on a digital transmitter circuit. The sensor mask design and passivation are currently being tuned to optimize long-term stability, repeatability, and precision before implantation in vivo. Conclusion: We have designed, fabricated, and tested a miniaturized sensor which detects physiologically relevant mechanical strains across the fusion gap to monitor the success of bone fusion.

135. Is Corticosteroid Treatment Associated with Sleep Related Breathing Disorders in Duchenne Muscular Dystrophy? A Preliminary Study

Karroum, Elias; Malik, Manisha; Phan, Han

Introduction: Duchenne Muscular Dystrophy (DMD) is an X-linked neuromuscular disorder due to mutations in the dystrophin gene. This results in progressive muscles degeneration leading to severe

ambulation restriction, cardiopulmonary insufficiency and death. Corticosteroids remain the only recommended therapy with established results, but have many adverse effects, including weight gain, cataracts, and glucose intolerance. Weight gain in DMD patients along with their weakened upper airway and respiratory muscles can result in sleep disordered breathing.Objective: To study the impact of corticosteroids treatment on sleep disordered breathing in DMD patients. Methods: Forty-four DMD patients (age = 14.3±3.5; BMI z-score = 0.1± 2.0; 23% ambulatory) were retrospectively studied based on treatment (n = 18) or not (n = 26) with corticosteroids. We collected polysomnographic data on total sleep time, sleep efficiency, arousals/hour of sleep, percentages of rapid eye movement (REM) and supine sleep, and respiratory measures including: apneas-hypopneas/hour of sleep (overall/during supine sleep/during REM sleep/strictly obstructive/strictly central), oxygen desaturations/hour of sleep, hypoxic burden (% SpO2<90%), nadir SpO2, and hypercapneic burden (% PCO2> 50 mmHg). Results: Patients on corticosteroids were more ambulatory (44% vs. 8%, p=0.008). They had similar obstructive sleep apnea (50% vs. 65%, p=0.3) and hypoventilation (6%vs.8%, p=1.0) prevalence's compared to patients not on corticosteroids. The also had a lower hypoxic burden (0.01±0.03% vs. 0.3±0.9%, p=0.04) and tendency for a lower hypercapneic (3.7±11.5% vs. 6.8±19.2%, p=0.06) burden and percentage of supine sleep (52±39% vs. 70±38%, p=0.07). Summary/Conclusion: These preliminary data do not suggest a clinically significant association between corticosteroids treatment and sleep disordered breathing in DMD patients.

136. Re-Visiting the Neurogenic Potential of Radial Glial Cells during Embryogenesis
Kuan, Chia-Yi; Huang, Henry; Chen, Hong-Ru; Lee, Jolly; Sun, Yu-Yo; Rakic, Pasko

Objectives: Radial glial cells (RGCs), once thought to be astrocytic precursor and a passive scaffold for cortical neuronal migration, are currently hailed as the principal neural progenitors in mammals. However, this assertion is largely based on in-vitro data and fate-mapping studies using transgenic mice that express an inducible Cre recombinase under the human glial fibrillary acidic protein promoter (hGFAP-CreER mice). Because these approaches may introduce artifacts, we seek to re-evaluate the neurogenic potential of RGCs using more stringent transgenic mouse lines. Methods & Results: We report three sets of results. Frist, in contrast to hGFAP-CreER mice, the mouse GFAP promoter-directed Cre driver line (mGFAP-Cre) failed to label the forebrain neurons but only some cerebellar Purkinje neurons in fate-mapping study. This finding raises concern on the specificity of hGFAP-CreER mice in previous studies. Second, we compared NestinCreER and BAC GLAST-CreER mice in multiple fate-mapping conditions, including tamoxifen induction at E10, E12, or E14 followed by E18 analysis, as well as, E14 or E18 tamoxifen-induction and P30 chase. These fate-mapping studies labeled cortical projection neurons, interneurons, astrocytes, and oligodendrocytes in the NestinCreER mice, as expected for common neuroglial progenitors. In contrast, the identical fate-mapping conditions in GLAST-CreER mice failed to label cortical projection neurons, but only RGCs undergoing astrocytic transformation in E18 and astrocytes plus few interneurons in P30 brains. However, postnatal newborn neurons in the rostral migratory stream were labeled in GLAST-CreER mice in all fate-mapping conditions. Finally, we tested if inhibition of the Notch signaling pathway at E15-16 using DAPT, a potent gamma-secretase inhibitor, can de-inactive the neurogenic activities of RGCs, as has been suggested. To our surprise, despite marked reduction of the mRNAs of Notch target genes, the DAPT treatment at E15-16 still failed to label cortical projection neurons in GLAST-CreER mice, further questioning the neurogenic potential of RGCs. Conclusions: Our results challenge the specificity of transgenic mouse lines used in previous studies to

support the neurogenic potential of RGCs. Our results suggest that RGCs have little, if any, contribution to cortical projection neurons, but may give rise to postnatal neural progenitors.

137. Beyond Microglia: Blood-Borne Monocytes Amplify Inflammation and Transform into Microglia after Neonatal Brain Injury in Rodents

Kuan, Chia-Yi; Sun, Yu-Yo; Lee, Jolly; Chen, Brandon; Huang, Henry; Archer, David R.; Anthony, Neil

Objectives: The brain resident microglia have a mesodermal origin, but they receive contributions by blood-borne monocytes in the neonatal period remains a contentious issue. Here we use transgenic monocyte- and microglia-reporter mice and adoptive transfer of genetically marked monocytes to investigate this issue. We hypothesize that (1) monocytes convert to microglia prenatally, but stop doing so soon after birth in normal conditions; (2) infection-sensitized neonatal hypoxic-ischemic (HI) injury attracts the influx of blood-borne monocytes, which amplify inflammation and transform into the brain resident microglia. Methods & Results: We report five sets of results. First, in E17 bitransgenic CCR2-RFP; CX3CR1-GFP embryos, monocytes (RFP+), microglia (GFP+), and RFP/GFP-double-positive cells were detected at specific locations between the choroid plexus and subcortical white matter. Time-lapse imaging in E17 brain slices indicated the monocyte-to-microglia conversion. Second, in postnatal P6 brains, EdU+ amoeboid microglial cells (AMC) at the classic “fountains of microglia” sites are exclusively CX3CR1-GFP-positive, but CCR2-RFP-negative cells, suggesting greatly attenuated monocyte influx after birth. Third, in P10 bitransgenic mice, a large number of CCR2-RFP+ monocytes and CCR2-RFP+/CX3CR1-GFP+ hybrid cells were found in the ipsilateral hemisphere of LPS (lipopolysaccharide)/HI-injured brains. The monocytes and monocyte-derived cells express pro-inflammatory cytokines (e.g. IL-1β and TNFα). Fourth, CCR2-KO mice showed near-absence of monocyte influx and greatly attenuated inflammatory responses to LPS/HI brain injury, leading to lesser brain tissue loss. Finally, intravenous adoptive transfer of actin-GFP+/CCR2-RFP+ monocytes to LPS/HI-injured P10 wild-type mice, GFP+ monocytes quickly down-regulated the CCR2-RFP expression, but transformed into AMC-like cells at 3 day, and microglia-like cells at 7-day recovery. Conclusions: Together, these results suggest that the blood-borne monocytes contribute to inflammatory responses in neonatal infection/HI injury, and likely transform into (pathological?) microglia, whose impacts on neural development are yet to be determined.

138. Osteopontin as a Blood Biomarker in Hypoxic-Ischemic Encephalopathy and Traumatic Brain Injury

Kuan, Chia-Yi

Objectives: Disease-associated blood biomarkers are invaluable tools for clinical management of neurological disorders, including hypoxic-ischemic encephalopathy (HIE) and brain trauma. Past research examined the utility of individual candidates, but few had used proteomic methods. We hypothesize that subject homogeneity in animal models may assist the discovery of brain-injury biomarkers using proteomic methods. Further, there may be common biomarkers for HIE and brain trauma due to shared mechanisms. Methods & Results: We report 4 sets of results. First, using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and bioinformatics, we found 16 plasma proteins with altered expression in neonatal mice 24 h after hypoxia-ischemia (HI) or endotoxin-sensitized HI injury, but

not endotoxin-challenge alone (n=3 for each group). Immunoblotting validated two up-regulated plasma proteins, including Osteopontin (OPN) that has been shown up-regulated in the brain after HI injury, but its expression level in the blood has not been studied before. Second, we showed that the induction of plasma OPN after HI is more sensitive than the blood glial fibrillary acid protein (GFAP) and matrix metalloproteinase 9 (MMP-9) levels. The plasma OPN concentrations tightly correlate with the extent of brain damage. Brain injection of endotoxin stimulates microglia and leads to elevation of the blood OPN protein levels. Third, we confirmed the induction of both brain and blood OPN levels after experimental head trauma in rats. Finally, we compared the blood OPN levels between 23 children (average=7.7 y/o) suffering from severe traumatic brain injury (Glasgow Coma Score < 8, mean GCS = 4.6) and 11 children (average=7.3 y/o) with mild trauma (GCS > 13) at admission and 24, 48, or 72 h in hospital if available. At admission, the two groups had similar plasma OPN levels (330 ng/ml in mild TBI and 462 ng/ml in severe TBI, p=0.11 by t-test). However, the plasma OPN levels in the severe TBI group rose to 582 ng/ml at 24 h (p=0.039 to admission), 657 ng/ml at 48 h (p<0.001 to at admission), and 947 ng/ml at 72 h (, p<0.0001 to admission). Conclusions: These results suggest that the microglia respond to hypoxic-ischemic or traumatic injury with induction of osteopontin, which in turn is released to the blood and serves as a biomarker of brain damage. Further studies are warranted to examine the plasma OPN levels over a longer duration in a larger patient cohort with correlation to clinical outcomes to assess this novel biomarker for brain injury.

139. Humanized Sickle Mice as an Experimental Model for Hemolysis-Endothelial Dysfunction Subtype of Stroke in Sickle Cell Disease (SCD)

Kuan, Chia-Yi; Sun, Yu-Yo; Lee, Jolly; Chappa, Prasanthi; Jiang, Rong; Wagner, Mary B.; Joiner, Clinton H.; Archer, David R.

Objectives: Complications in Sickle Cell Disease (SCD) are classified into viscosity-vaso-occlusion and hemolysis-endothelial dysfunction subtypes, which correspond to large overt stroke and silent cerebral infarct, respectively. There are major gaps in the current stroke management for SCD children, and humanized sickle mice (knock-in/out mice that express the human α, γ, and sickle-β hemoglobin genes) rarely exhibit spontaneous stroke and are too fragile to endure experimental stroke. Here we test the hypothesis that humanized sickle mice have hemolysis-endothelial dysfunctions in cerebral blood vessels and a higher sensitivity to hypoxia-ischemia. Methods & Results: We report three sets of results. First, 6-month-old sickle mice of the SS genotype (βS/βS) have a higher peak systolic velocity (PSV) and a greater resistive index (RI) despite indistinguishable histology in the common carotid artery compared with AA (βA/βA) and AS (βA/βS) mice (n=7-9 for each genotype). This finding suggests increased vascular flow impedance, which is supported by ectopic expression of P-Selection and plasminogen activator inhibitor 1 (PAI-1) in cerebral blood vessels of SS mice. Second, 6-month-old SS mice endure 20-min transient hypoxia-ischemia (tHI), but show prolonged cerebral hypo-perfusion and extensive fibrin deposition at 2 h, as well as, a much higher mortality rate at 24 h recovery (n=8 for each genotype). Finally, 3-month-old SS mice show motor deficits and 60% cumulative mortality following unilateral carotid ligation and 3 daily exposures to 15 min hypoxia (repetitive mild hypoxia-ischemia, rmHI), while none of AA mice exhibit such responses (n=10 for each genotype). Histopathological analysis of the surviving SS mouse brains also reveal pockets of astrogliosis and fibrin deposition. Conclusions: Our results suggest that while humanized sickle mice rarely develop spontaneous stroke or exhibit proliferative vaso-occlusion vascular pathology, they mimic hemolysis-endothelial dysfunctions seen in SCD patients.

Furthermore, our results suggest two experimental models—transient hypoxia-ischemia (tHI) and repetitive mild hypoxia-ischemia (rmHI)—which can be used to investigate novel strategies for primary prevention and acute therapy of ischemic stroke in SCD.

140. YAP-Mediated Metabolism and Mitochondrial Morphology in Medulloblastoma
Malhotra, Anshu; Dey, Abhinav; Kenney, Anna M.

Sonic hedgehog (Shh) signaling is closely coupled with bioenergetics of medulloblastoma, the most common malignant pediatric solid tumor. Shh causes deregulation of mitochondrial biogenesis by suppressing mitofusins, leading to fragmented mitochondria in vitro as well as in SmoA1 mouse medulloblastomas (MB) in vivo. Ectopic expression of mitofusins restores mitochondrial fusion accompanied by a rescue in proliferation to the non-proliferative phenotype. YAP (Yes-Associated Protein) acts downstream of the Shh pathway to regulate proliferation in tumor cells. We have observed that YAP also regulates the expression of mitofusins, OPA1 and the complexes of the oxidative phosphorylation. This study investigates the role of YAP in deregulating cellular energetics to support the actively proliferating tumor cell. Our goal is to determine if manipulating YAP-mediated mitochondrial dynamics can restore the metabolic profile of tumor cells to that of non-transformed, non-proliferating cells, thus suggesting a potential novel treatment paradigm that may reduce or eliminate the requirement for high dose radiation.

141. Effects of Vagus Nerve Stimulation on Sleep Related Breathing Patterns in Epilepsy Patients in the Pediatric Population: A Retrospective Chart Review
Malik, Manisha; Karroum, Elias; Phan, Han

Introduction: Epilepsy is the most common chronic neurologic disease and impacts health, development, education, and quality of life. Incidence of pediatric epilepsy is 5 to 8.4 per 1000 patients, one third of those are medically refractory. Alternative therapy to medication in epilepsy is vagus nerve stimulator (VNS), ketogenic diet and epilepsy surgery. VNS uses a pulse generator to send mild electrical stimulations to the vagus nerve and helps calm down the irregular electrical brain activity leading to seizures. Stimulation of vagus nerve afferent fibers can also cause vocal cord dysfunction, laryngeal spasm and dyspnea. VNS causes a decrease in respiratory amplitude, decrease in tidal volume and decrease in oxygen saturation during periods of device activation. Patients with VNS can have central apneas, obstructive hypopneas, and obstructive apneas. Published study shows OSA after VNS placement, however it accounted for patients 21-58 years. The purpose of our study is to examine the relationship between VNS and prevalence of OSA in intractable epilepsy patients by analyzing polysomnogram (PSG) data for these individuals in the pediatric population. Materials and methods: A retrospective chart review was conducted from Children's Healthcare of Atlanta Pediatric Epilepsy program. We identified 17 children who had VNS implantation and PSG performed from 2009-2015. Post-VNS PSG variables were extracted and analyzed. AHI represents average number of apnea or hypopnea events per hour during sleep and AHI >1/hour meets the diagnostic criteria for OSA. A similar cohort of 16 children from the intractable epilepsy program was identified, who did not have VNS and used as control group. Results: Ages ranged from 2 to 20 years (M = 11.6 SD = 5.0) and our sample consisted of 7 boys and 10 girls in the study group and 11 boys and 8 girls in the control group. 100% had

intellectual disability. 82.6% of cases and 85% control were non-ambulatory. VNS group had higher AHIs as compared to non-VNS group (average AHI for VNS = 4.44, average AHI for non-VNS = 1.75), t(25) = -1.7, p = 0.05. Equal variances for these statistics could not be assumed. Conclusion: VNS is a common treatment for children with refractory epilepsy. Based on the finding of our small study, there is evidence demonstrating presence of OSA as apparent by higher AHIs in patients with VNS. PSG before implantation of VNS should be considered to identify patients with pre-existing OSA to prevent worsening of OSA.

142. All-In-One, Multiplexed On-Bead ELISA for Detection of Two Malarial Biomarkers
Markwalter, Christine; Ricks, Keersten; Bitting, Anna; Mudenda, Lwiindi; Wright, David

Malaria is a leading cause of death worldwide in children under five. Children bear most of the global malaria burden, comprising 70% of the estimated 500,000 deaths annually. Accurate diagnosis is important for identifying and treating malaria-infected patients, defining disease prevalence and distribution, as well as monitoring impact of interventions. Further, diagnosis and treatment of asymptomatic carriers are critical for eliminating the disease. Current antigen-detecting rapid diagnostic tests (RDTs) for malaria are unreliable in the asymptomatic regime (< 200 parasites/µl), and laboratory protein-based detection strategies, such as well-plate ELISAs, require 5 - 8 hours of incubation time and are limited to one analyte. To address this, we have developed a multiplexed, magnetic bead-based ELISA with incubation times totaling less than 1 hour and single parasite/µl detection limits rivaling those of well-plate ELISAs. This multiplexed assay detects two malarial biomarkers: (1) *Plasmodium* lactate dehydrogenase (pLDH), which is detectable for all five species of malaria, and (2) histidine-rich protein II (HRPII), present only in *P. falciparum* infections. In this assay, magnetic particles functionalized with antibodies specific for pLDH and HRPII are added directly to lysed whole blood samples along with detection antibodies with distinct enzymes for each biomarker. Sandwich complexes for pLDH and HRPII form on the surfaces of the magnetic beads, which are washed and sequentially re-suspended in detection enzyme substrates for each antigen. Detection of both biomarkers is advantageous because it avoids false-positives due to slow HRPII clearance and allows for differentiation between *falciparum* and non-*falciparum* infections, ultimately informing treatment. With these advantages, as well as high sensitivity and detection limits in the single parasite/µl regime, the developed multiplexed assay for pLDH and HRPII is an attractive alternative to well-plate ELISAs and a promising detection strategy for an elimination setting. Further, the modularity of the multiplexed on-bead ELISA makes it applicable to any series of infectious disease biomarkers for which there are antibody pairs available.

143. The Functional Role of Tumor-Associated Macrophages in Medulloblastoma
Maximov, Victor; Alikhanyan, Kristina; Hambardzumyan, Dolores; Kenney, Anna M.

Medulloblastoma (MB) is the most common malignant pediatric solid tumor of the nervous system, arising in the cerebellum. The current standard of care combines surgical resection with chemo- and radiotherapies, resulting in a 5-year survival rate of approximately 70%. However, survivors are frequently left with life-long side effects such as hormonal dysfunction, seizures, intellectual impairment, and susceptibility to cancer recurrence, which is often lethal. MBs can be divided into 4 subtypes according to unique gene expression patterns, genomic abnormalities, and histological traits. One such type, the Shh

subgroup, features aberrant activity of the Shh pathway, a critical developmental pathway in the normal cerebellum. Currently, inhibitors for this pathway do exist, but their use is associated with development of resistance, indicating that novel methods of treatment are needed. The recent literature for novel brain cancer treatment development has been increasingly focused on targeting the tumor microenvironment, as it has been shown to play a significant role in tumor initiation and progression in glioblastoma (GBM) models. One of the key components of the tumor microenvironment is the population of Tumor-Associated Macrophages (TAMs), which comprise as much as 30% of the total number of cells in the tumor. According to the literature, there are two distinct types of TAMs that could be present in brain tumors: resident microglia and bone marrow derived macrophages (BMDMs) infiltrating from blood stream. It was shown that these TAMs could play a significant role in brain tumor growth and development, by inducing angiogenesis and suppressing other immune cells functions. A recent publication in Clinical Cancer Research studied TAMs in four different subtypes of human MBs and revealed a great accumulation of TAMs specifically in the Shh subgroup, but there is no literature available on the role of TAMs in MB. The main goal of the present study is to investigate the role of TAMs in the Shh MB. We have found an elevated number of activated TAMs in our mouse model for the Shh subgroup of MB when compared to the normal cerebellar tissue. Using in vitro cell culture models, we investigated the effect of proteins secreted by MB tumor cells on the gene expression and polarization of microglia and BMDMs. We then evaluated the effect of activated microglia and BMDMs on the proliferation rate of MB tumor cells. The data obtained suggests that TAMs play a significant role in tumor growth and progression through yet-to be-determined mechanisms.

144. Electrically-Induced Calcium Handling in Cardiac Progenitor Cells

Maxwell, Joshua; Wagner, Mary B.; Davis, Michael

The discovery of a resident population of cardiac stem cells known as cardiac progenitor cells (CPCs) has raised the question of the mechanisms involved in regenerative properties of this niche of cells. The physiology of human CPCs is not well understood, and a thorough understanding of the Ca2+ handling ability of these cells is crucial in order to fully realize their regenerative potential. Therefore, to characterize the mechanism of electrically-induced Ca2+ handling in human CPCs, we have used cells loaded with fluo-4 calcium dye and imaged using confocal microscopy to determine the spatial and temporal profile of cytosolic Ca2+ upon electrical stimulation. Upon activation of electrical stimulation, a large increase in cytosolic Ca2+ was observed followed by oscillations of the cytosolic Ca2+. The electrically-induced Ca2+ oscillations were found to be dependent on the activity of L-type Ca2+ channels (LTCC) and the presence of extracellular Ca2+. Furthermore, these oscillations could be abolished by pre-treatment of the cells with the inositol 1,4,5-trisphosphate receptor (IP3R) antagonist 2-APB. High-speed confocal imaging of the electrically-induced Ca2+ oscillations in hCPCs revealed that these cells oscillate in a Ca2+ wave-like fashion with activity originating in isolated regions of the cell and propagating throughout the cytosol and nucleus. In conclusion, we propose that Ca2+-dependent regulatory mechanisms can be exploited to influence their differentiation potential leading to improved function in cardiac regenerative medicine.

145. Appropriate Use of REDCap in Clinical Research Studies

McCracken, Courtney; Travers, Curtis; Kelleman, Michael S.; Gillespie, Scott; Leong, Traci

Introduction: Pediatric investigators are required to collect, input and manage data arising from clinical research studies. In previous years, clinicians were limited to Microsoft Excel spreadsheets or homegrown Access databases that lacked standardization and portability. In recent years, Emory and CHOA have launched REDCap (Research Electronic Data CAPturing system) to allow for web-based data entry of clinical data. While this system offers substantial advantages over preexisting data structures, it is limited in its capability to handle large volumes of data and/or complex study designs. Our primary objective is to describe appropriate and inappropriate uses of REDCap in the clinical research setting. Methods: Recommendations for the use of REDCap were obtained from the pediatric biostatistics core's experience. The core has built and maintained over 50 REDCap databases and has extensive knowledge with the limitations of REDCap in pediatric research. Results: REDCap is a powerful tool offering online databases that are easy to design, implement, and manage. It can handle retrospective, cross-sectional and prospective studies with a pre-defined number of events (e.g., study visits, lab draws). Since clinical trials must follow a strict protocol and have pre-defined time points, these studies are well suited for REDCap and can be expanded to include multiple institutions. Small studies or studies collecting data on patients over a finite period of time with a limited number of variables are also appropriate. Despite REDCap's versatility, there are a number of study formats that are not suited for REDCap. Studies involving an unknown number of events, duration of follow-up, or frequent and/or unstructured visits/variables are not appropriate. For some studies, there are challenges when capturing one-to-many events (such as adverse events or medication changes arising from a single patient), requiring a secondary REDCap project to accommodate different data structures. REDCap is not designed to replace EHR or provide a mechanism for long-term surveillance of a cohort. It is limited in its ability to pre-populate data fields, carry forward data across events, and handle multiple repeating events without a predefined data structure. Conclusions: REDCap is a powerful tool that has improved our ability to collect and store data for clinical research. Despite its easy point and click interface, there are challenges to building a data structure that is suitable for many research studies. Consulting a biostatistician prior to designing a data collection tool is the appropriate first step.

146. Satisfaction, Pain, and Bleeding- A Comparison of Two Different Ear Curettes

Mohammed, Anaam; Baxter, Amy; Spandorfer, Philip

Objectives: To determine whether a novel flexible ear curette was preferred to a traditional stiff curette, and to analyze differences in clinical outcomes. Methods: Patients between 0 to 21 years were randomized to EasiEar™ or Bionix™ curettes and further block randomized by age. Patient pain, nurse practitioner satisfaction, and bleeding during examination were determined. Results: 82 patients were enrolled. There was no significant difference in patient pain (0-5: p=0.4273; 6-21: p=0.6009). Nurse practitioners significantly preferred EasiEar™ (0-5: p <0.0001; 6-21: p=0.0002). Conclusions: Although EasiEar™ curettes do not significantly decrease patient pain, nurse practitioners were significantly more satisfied with them.

147. 50-Valent Inactivated Rhinovirus Vaccine is Broadly Immunogenic in Rhesus Macaques
Lee, Sujin; Nguyen, Min Trang; Currier, Michael; Jenkins, Joe; Strobert, Elizabeth; Kajon, Adriana; Madan-Lala, Ranjna; Bochkov, Yury; Gern, James; Roy, Krishnendu; Lu, Xiaoyan; Erdman, Dean; Spearman, Paul; Moore, Martin

As predominant etiological agent of the common cold, human rhinovirus (HRV) is the leading cause of human infectious disease. HRV also causes pneumonia hospitalizations in children and adults and exacerbations of asthma and chronic obstructive pulmonary disease (COPD). Decades ago, researchers identified inactivated HRV as a protective vaccine, defined virus-neutralizing antibodies (nAb) as a correlate of protection, and estimated duration of immunity. However, co-circulation of many HRV types discouraged vaccine efforts. Two 10-valent inactivated HRV vaccines were tested, but nAb were induced to only one-third of the input strains, though the average input virus titer in those 10-valent vaccines was low. We tested the hypothesis that increasing virus input titers in polyvalent inactivated HRV vaccine will result in broad nAb responses. Here we show that serum nAb against many rhinovirus types can be induced by polyvalent, inactivated HRVs plus alhydrogel (alum) adjuvant. Using formulations up to 25-valent in mice and 50-valent in rhesus macaques, HRV vaccine immunogenicity was related to sufficient quantity of input antigens, and valency was not a major factor for potency or breadth of the response. We for the first time generated a vaccine capable of inducing nAb responses to numerous and diverse HRV types.

148. Racial Disparities in the Clinical Characterization Profiles of Individuals with Autism Spectrum Disorder
Reid, Morganne; Moriuchi, Jennifer; Klin, Ami; Saulnier, Celine

Studies examining differences between African-American and Caucasian individuals with Autism Spectrum Disorder (ASD) have yielded equivocal findings. African-American individuals with ASD are more likely to have developmental delays yet are less impaired in adaptive and executive functioning and exhibit less externalizing and stereotypical behavior compared to Caucasian peers. Factors underlying this discrepancy remain unclear but likely contribute to previous findings of delays in diagnosis, misdiagnosis of disruptive behavior disorders, and reduced access to care for African-American individuals with ASD. To help address this discrepancy, the current study compares phenotypic profiles of African-American and Caucasian school-age individuals with ASD on measures of cognition, adaptive functioning, and diagnostic symptomatology. Participants included 155 individuals with ASD (95 African-American, 60 Caucasian) ranging in age from 3 to 18 years (mean=8.3, SD=3.9). Measures included the Differential Ability Scales, 2nd Edition (DAS-II); Autism Diagnostic Observation Schedule, 2nd Edition (ADOS-2); and Vineland Adaptive Behavior Scales, 2nd Edition (Vineland-II). Analyses using a preliminary sample revealed significant racial differences. African-American individuals scored significantly lower than Caucasian individuals across measures of verbal, nonverbal, and overall cognitive ability [DAS-II GCA: $p<.001$]. African-American individuals with ASD also exhibited significantly greater restricted and repetitive behavior compared to Caucasian peers [ADOS-2 RRB: $p<.01$]. Given the significant difference in cognitive ability, analyses were repeated in a subset of participants without cognitive impairment (DAS-II GCA>70). Even within this subset without cognitive impairment, significant differences remained across measures of cognitive ability; African-American individuals continued to score lower than Caucasian peers. Differences in restricted and repetitive behavior were no longer

significant. However, African-American individuals without cognitive impairment had significantly lower adaptive socialization scores, even after controlling for cognitive ability [Vineland-II Socialization: p<.01]. In summary, we found that African-American individuals with ASD exhibited significantly lower cognitive ability and higher levels of restricted and repetitive behaviors than Caucasian peers. Only 24% of the Caucasian sample had cognitive scores below 70 compared to 56% of the African-American sample. When limiting the sample to individuals without significant cognitive impairment, African-American individuals with ASD still exhibited lower cognitive ability in addition to greater adaptive socialization deficits. African-American individuals' adaptive socialization deficits remained even after controlling for cognitive ability and even in the absence of differences in ASD symptomatology. These findings help clarify the discrepancy in clinical profiles of African-American and Caucasian individuals with ASD, highlight areas for targeted intervention, and raise questions about how to most appropriately conceptualize 'level of functioning.'

149. Genome-wide Association Identifies African-Specific Susceptibility Loci in African Americans with Inflammatory Bowel Disease

Okou, David; Cutler, David; Chopra, Pankaj; Prince, Jarod; Venkateswaran, Suresh; Zwick, Michael; Kugathasan, Subra

The inflammatory bowel diseases (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory gastrointestinal conditions associated with significant morbidity and a rising prevalence in all populations. More than 200 susceptibility loci have been identified in European ancestry populations and a handful has been identified as Asian-specific. African Americans (AAs) suffer the same disease burden as Europeans. They have a higher risk for developing disease complications and worse disease outcome but no African-specific loci have been established. We hypothesized that high-density GWAS of IBD in AAs could identify population specific variants and expose novel disease mechanisms. We combined two, previously unpublished, AA genome-wide associations scans (GWAS) comprising 2345 IBD cases recruited from multiple centers across the US and 5002 controls derived from the Database of Genotypes and Phenotypes (dbGaP) and Kaiser Research Program on Genes, Environment and Health (RPGEH). We identified a novel Sub-Saharan African-specific UC locus (19q31) that contains 5 SNPs (monomorphic in European and other populations) at the ZNF649 gene. ZNF649 influences the NFκB pathway and TNF activation. We provide the first association from GWAS of BTNL2 (HLA region) to IBD in AAs (BTNL2 was associated with IBD in Caucasians and CD in Koreans by deep sequencing only). We observed multiple African-specific SNPs with suggestive evidence ($5x10^{-8}<p<6.5x10^{-6}$) of association at the TNC gene for UC (the top SNP has OR=2.06 and $P=3.68x10^{-6}$) and IBD (the top SNP has OR=1.65 and $P=9.6x10^{-8}$); and within the CXCR6 for CD (OR=0.6, $P=6.94x10^{-7}$). Additionally, we show that many loci originally found in Europeans are shared across ancestries. In this first AA GWAS for IBD, we have identified a novel UC locus on chromosome 19 and further expanded our understanding of the contribution of the HLA in this population. We provide evidence for the first genome-wide significant African-specific UC locus at the ZNF649 gene, the function of which seems biologically relevant to UC pathophysiology. We show varying contribution of the HLA region to UC in AAs and evidence of genetic heterogeneity underlying UC between AAs and European populations. A high proportion of IBD loci found in Europeans are shared across ancestries, in support of shared pathogenic mechanisms across different ethnicities.

150. Measuring Early Social Development and Affect in Infants and Toddlers during a Brief Play Interaction: Development of an Online Interactive Parent Screener

Ousley, Opal; Li, Gaizhi

Objective: The evaluation of early social responsiveness may allow detection of altered neurodevelopment that precedes or co-occurs with global developmental delay, language delay, or autism spectrum disorder. This study will report the results of a brief interactive behavioral assessment using data collected via in-person as well as online assessments. Methods: This study includes children 15-36 months of age, recruited from the community without regard for the presence of a developmental delay. For this study, children participate in an interactive assessment either in-person or via an online format. The assessment is completed in approximately 4 minutes and comprises 5 play activities: 1) smiling and saying hello, 2) ball play, 3) looking at a book, 4) engaging in a silly activity (i.e., pretending a book is a hat), and 5) tickling. Child responses (e.g., smiling, making eye contact, and ease of social engagement) are coded to create activity scores as well as a total score. Higher scores represent poorer social responsiveness. Each activity is also scored using a 6-point scale code the child's affective valence (e.g., "affect score"), with a 6 representing the most positive affect. Results: All the in-person assessments have been completed (n=68) and the online data collection is ongoing. For the in-person assessments, the mean age equals 20.79+/-3.92 and the mean activity scores are: 1) 1.64+/-1.24 (range = 0 to 4); 2) 1.98+/-1.50 (range = 0 to 6); 3) 4.26+/-2.13 (range = 0 to 8); 4) 1.14+/-1.17 (range = 0 to 4); 5) 0.99+/-1.54 (range = 0 to 5). The mean total score equals 10.03+/-4.80. The mean affect scores for each activity are: 1) 3.28+/-0.15; 2) 4.97+/-0.16; 3) 3.51+/-0.17; 4) 3.67+/-0.15; 5) 5.04+/-0.15. We will report the same data as above for the online data collection group. We will also match children on age and gender, across the two samples, and compare the scores from the in-person versus online assessments, using t tests, in order to examine the validity of online data collection. Further, we will compare groups of children based on parents' *a priori* concerns about their child's development. Conclusions: The planned data collection will provide us with information about the validity of using interactive parent report via an online data collection format. If we are successful, this methodology could result in a paradigm shift in how we track social behavioral development and could lead to large scale or whole population screening.

151. Somatic Physiology in the Behavioral Expression of 22q11.2 Deletion Syndrome

Pearce, Brad; Weng, Lei; Ousley, Opal; Morgan, Kristiana; Kobrynski, Lisa; Oster, Matthew E.; Cubells, Joseph; Coleman, Karlene

The 22q11 deletion syndrome (22q11DS) is a chromosomal disorder involving a micro-deletion on one copy of chromosome 22. Common features of this disorder include cardiac defects, endocrine dysregulation, and immune abnormalities. In this population, there is a high risk for schizophrenia, and 14% to 50% meet criteria for an Autism Spectrum Disorder (ASD). However, it is unclear why some individuals with 22q11DS meet diagnostic criteria for ASD, while others with the same deletion develop other problems (peer-related social problems in the absence of ASD), and still others exhibit variable degrees of learning difficulties that can be quite mild, and hence allow these people to attend college and hold steady jobs. We hypothesize that the differences between individuals in the character or severity of somatic illnesses (immune, cardiac, endocrine) may help determine individual differences in the neuropsychological features of 22q11DS. To test this hypothesis, we are using the robust medical record

and lab data collected at Emory and Children's Healthcare of Atlanta (CHOA) under IRB#00045086. In 1995, Karlene Coleman and Lisa Kobrynski founded the Emory-Children's Healthcare of Atlanta Southeastern Regional Center of Excellence for 22q11DS. Accordingly, we created a comprehensive database, the Southeast Regional Phenotypic 22q11DS (SERPh22) database that integrates detailed clinical and physiological data with psychological test scores in toddlers, children, and adults with 22q11DS. The SEPRh22 database has over 4000 data entry fields covering broad disease history categories (obstetric, cardiac, immune, endocrine, craniofacial) and detailed granular level information such as longitudinal laboratory values, surgical notes, biometrics, and medical complications. We also have detailed fields for psychological tests scores including tests for infants and toddlers e.g. Communication and Symbolic Behavior Skills test (CSBS), children (ADOS, ADI), and adults. Currently, the dataset consists of 713 individuals with 22q11DS, and 294 have blood biomarker data such as CD4 cell counts. Using the SERPh22 resource, we have recently reported that lower serum calcium was associated with significantly greater impairment in the CSBS test scores (Muldoon et al. 2015). Another area of interest is the role of immune abnormalities in the expression of ASD. Using regression modeling we found that among patients with 22q11DS, levels of serum IgG were significantly negatively correlated with Aberrant Behavior Checklist (ABC) scores (β=-0.029, SE=0.01, age Adjusted R2= 0.32, P=0.04). These data indicate that the severity of immune dysfunction (IgG deficit) may be one factor associating with neuropsychological outcome in this high-risk patient group.

152. Retrospective Comparative Chart Review of Spinal Muscular Atrophy
Phan, Han; Bassell, Julia

Spinal muscular atrophy (SMA) is an autosomal recessive disease that causes degeneration of motor neurons in the spinal cord, leading to hypotonia and progressive muscle weakness. The incidence of SMA is 1 in every 10,000 births, and is the most common genetic cause of infant mortality. There is currently no cure for SMA, and the average age of death in children with SMA type I is 2 years and is often resulted from respiratory complications. Spinal muscular atrophy is a large cohort in our Emory MDA clinic, comprising about 20% of our clinic patients. Over the past year, we have observed a disturbing trend in our SMA patients who have succumbed to death at a startling early age as compare to the published data. Our study seeks to better understand varying factors of respiratory care and treatment that affect the survival of SMA type I patients and identify potential factors that impact the survival age of these patients in order to improve mortality in this particular population. This retrospective chart review focused specifically on respiratory care, intervention and pulmonary outcome in addition to investigating the delay of symptom onset and diagnosis in these patients. By comparing our patient population with the current published data (data obtained from Finkel et. al 2014), we found that our age of symptom onset and age of clinical diagnosis was significantly later, and our time at which noninvasive and invasive ventilator support was earlier. In addition, our time at which GI tube placement was significantly later. This data was compared for both SMA type 1 and SMA type II. We also found that our average age of death for SMA type I was earlier. We had 11 patients with SMA type I and 16 children with SMA type II. By analyzing methods of clinical diagnosis which created the initial set back in diagnosis and therefore effects on treatment, we were able to hypothesize possible ways to close the diagnosis time and to improve long term support by better understanding the varying factors of respiratory care.

153. Importance of Polysomnograms in Children with Spinal Muscular Atrophy
Phan, Han; Bassell, Julia

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disorder with a prevalence of 1 in every 10,000 births, and is the most common genetic cause of infant mortality. Because of the muscle weakness in SMA, children have very weak intercostal muscles, bulbar muscle weakness, and a soft and flexible chest wall that creates the signature collapsed or bell shaped chest wall that can be seen in these children. This muscle weakness leads to an impaired cough resulting in poor clearance of lower airway secretions, hypoventilation during sleep, chest wall and lung underdevelopment, recurrent infections (aspiration pneumonia) that exacerbate muscle weakness and the integrity of the lung parenchyma; all these sum to progressive respiratory failure. Because death is commonly associated with respiratory problems, it is imperative to better assess what tools can be used to help respiratory function and treatment. Even though the polysomnogram is the gold standard when it comes to respiratory function, there has not been adequate research in regards to sleep problems in children with SMA. We conducted a retrospective chart of the patient population at Emory University and quantified how many patients out of our total SMA type I and II populations had received a sleep study and the results of the sleep study. We found that only 45.5% of our SMA type I population and 50.0% of our SMA type II population had received a sleep study, even though the consensus statement for standard of care includes a recommendation for polysomnogram studies. We then investigated pulmonary function and age that noninvasive ventilatory support started with age of sleep study. Our study sought to better understand not only the effects of sleep problems in children with SMA but also unravel why the gap in implementation of sleep studies exists.

154. Does High Pulmonary Venous Forward-Reverse VTI Ratio Predict Postnatal Morbidity Related to Pulmonary Over-Circulation in the Fetus with Hypoplastic Left Heart Syndrome?
Posey, Jessica; Lindsey, Kristina; McGaughy, Falon; Friedman, Heather; Allen, Nicole; Pan, Anqi; Sachdeva, Ritu; Michelfelder, Erik

Background: In the fetus with hypoplastic left heart syndrome (HLHS) Doppler flow patterns in the pulmonary veins (PV) - specifically, a low PV forward/reverse velocity time integral ratio (FR VTI) - can reliably identify the fetus at risk for highly restrictive atrial septum. Conversely, it is unknown whether a high FR VTI may be predictive of pulmonary over-circulation, and its hemodynamic sequelae. The purpose of this study is to evaluate whether a high PV FR VTI has implications for pulmonary over-circulation and acidosis in the postnatal pre-surgical window. Methods: A retrospective review of all infants with prenatally diagnosed HLHS admitted at CHOA Egleston from 2004-2015 was performed. Fetal measurements of PV FR VTI ratio were recorded; subjects were grouped as low (<3), medium (3-10), and high (>10) PV FR VTI. Clinical and hemodynamic measures, including postnatal respiratory support, inotrope use, oxygen saturation, and acid-base status were compared between groups with low, medium, and high PV FR VTI. Results: A total of 43 eligible HLHS subjects were identified. Fetal PV FR VTI ranged from 1 to 70. There were significant differences amongst groups in level of pre-Norwood respiratory support, FiO2 level, and paO2, most markedly at 48 hours of life. On post-hoc analysis, only paO2 at 48 hours differed significantly between the medium and high FR VTI groups. There were no significant differences in inotrope requirements, peak lactate, or base deficit between groups at any postnatal time point. Conclusions: Though there are significant differences in postnatal cardiopulmonary

findings in neonates with varying PV FR VTI, our study results suggest that high FR VTI ratio is not associated with a higher incidence of inotrope requirement, acidosis, or increased respiratory support compared to fetuses with medium range PV FR VTI. While identifying the fetus with HLHS and low (<3) PV FR VTI has been shown to be an important clinical finding, it does not appear that identification of a high PV FR VTI adds clinically useful data to the management of the fetus with HLHS.

155. Reactive Oxygen Species Signaling in Shh Driven Medulloblastoma and Cerebellar Granule Neuron Precursors: The Nox4-Hif1a Axis

Potts, Chad; Eyrich, Nicholas; Kenney, Anna M.

Medulloblastoma is the most common solid malignant pediatric brain tumor. These tumors arise in the cerebellum and can be molecularly subdivided into 4 consensus subgroups, one of which is marked by amplification and activation of Sonic hedgehog (Shh) pathway components and downstream targets. This subclass is proposed to arise from oncogenic transformation of cerebellar granule neuron precursors (CGNPs), whose expansion during post-natal brain development is driven by and requires activation of the Shh pathway. These tumors often demonstrate similarities with normal cerebellar development at the molecular level, thus allowing us to use primary CGNP cultures as a model system for the Sonic hedgehog (Shh) driven subclass of medulloblastoma. In addition to mitogens driving proliferation, it has been shown in the past that low levels of intracellular reactive oxygen species (ROS) are required for proliferation, through mechanisms as diverse as inhibition of receptor tyrosine phosphatases, stabilization of proliferation proteins, and metabolite modification. We have observed a global increase in the cellular concentration of ROS in CGNPs that have been treated with Shh. This has led us to investigate potential sources and targets of these ROS. We carried out a qPCR screen for ROS-regulating proteins and found a significant induction of NADPH Oxidase 4 (Nox4) mRNA in Shh-stimulated CGNPs. The NADPH oxidases are a family of proteins originally associated with the oxidative burst in neutrophils wherein NADPH catalyzes the conversion of oxygen to superoxide that can destroy microorganisms during phagocytosis. More recently they've been discovered to generate ROS as a means of signaling modification and are particularly associated with inflammatory responses. Nox4 has not previously been studied in medulloblastoma. In addition, we observed high levels of Hif1a protein in proliferating CGNPs. HIF1a is classically thought of as a protein that only becomes active in low oxygen conditions to spur angiogenesis and switch the cell to anaerobic metabolism. Recently it has been implicated as part of the Warburg effect which causes a noted increase in glycolytic activity and relative decrease in oxidative phosphorylation in oftentimes normoxic cancer cells. We have previously shown that Shh-treated CGNPs and medulloblastoma cells are highly glycolytic. We only observed a slight up-regulation of HIF1a mRNA, suggesting a stabilization mechanism may underlie the elevation of protein. Interestingly, inhibition of NOX4 and ROS resulted in decreased levels of Hif1a. Thus, we hypothesize that HIF1a stabilization may result from modification of prolyl hydoxylases, which cause HIF1a degradation, by Nox4-mediated ROS generation.

156. Gene Identification through Genome Wide Association and Genotype-Serotype Correlation in Pediatric Ulcerative Colitis.

Prince, Jarod; Venkateswaran, Suresh; Dodd, Anne; Le, Khuong Uyen; Okou, David; Kugathasan, Subra

Background: Ulcerative Colitis (UC) denotes a phenotype of IBD where inflammation is localized to the colonic mucosa, and extends from the rectum proximally in varying extents. Identifying the susceptibility loci through GWAS has been extensively done in children with IBD, CD, but similar analysis has limited in Caucasian specific pediatric UC. Aim: Perform a GWAS through high density SNP chip and to examine the relationship of serological and genetic markers. Methods: Baseline data were obtained from children (4-17 years) in the prospective PROTECT Study: (DK 095745). Diagnosis of UC extending above the rectum was made using standardized criteria. For the genetic expression analysis 763 children with newly diagnosed with UC were selected and control samples were selected from UK Biobank. A dense genotyping was performed on this dataset through Axiom Analysis Suite. The GWAS study was performed on a dataset of 2565 samples (466 cases and 2099 controls). Serological markers were observed for pANCA, anti-CBir (anti-flagellin). Association was evaluated using chi square tests and t-tests. Results: Our study identifies genome-wide significant associations with 34 novel variants with the p-value of <= 5*10-8, among them 32 are HLA variants. Among the other ~300 variants identified as susceptible loci, two of them namely; RNF186 and GPR35 were also identified as genome-wide significant in the previous study performed on UC cohort. Of 422 children enrolled, 119 were <12 years and 303 were ≥12 years old. Among the 403 patients (95%), which are having serology; 262 were pANCA+ and 141 were pANCA- and; 79 were CBir+ and 324 were CBir-. The genetic results were correlated with the age and serological groups and identified highly associated genetic markers in each of the following groups; (i) the age between <12 vs ≥12 years identified NFKB1 and, IL23R genes, (ii) pANCA+ vs pANCA- identfied HLA-DRB1 and NFKB1 genes and, (iii) CBir+ vs CBir- identified POU5F1 and PLCL1 genes. Conclusions: Genetic predisposition to UC varies when stratified by age of onset, serological reactivity, and other stochastic factors. Previous studies have shown that pANCA and CBir are predictive serological markers in UC, and that the HLA region plays an instrumental role in UC, but our study is the first to look at genotype, serotype correlation in relation to pediatric onset UC. By using newly discovered genetic variants, serological reactivity markers, and phenotypic characteristics a better model can be developed for molecular sub-classification of early onset UC and disease prognostication.

157. Prodrug-Mediated Elimination of Tumorigenic Human Pluripotent Stem Cells using Antibody-Guided Virus-Like Particles

Preininger, Marcela K.; Crooke, Stephen; Jha, Rajneesh; Ding, Lingmei; Spearman, Paul; Finn, M.G.; Xu, Chunhui

Sensitization to prodrugs via transgenic expression of suicide genes is a leading strategy for selective elimination of potentially tumorigenic human pluripotent stem cells (hPSCs). However, since transgenic cellular modification poses the risk of deleterious mutagenesis, this study sought to establish a safer, alternative system for specific intracellular delivery of suicide-inducing macromolecules to viable hPSCs using virus-like particles (VLPs). Virus-like particles were engineered to contain cytosine deaminase (CD) enclosed in Qβ bacteriophage capsids and displaying surface IgG-binding ZZ domains (i.e. Qβ(ZZ)@CD). For hPSC targeting, Qβ(ZZ)@CD virus-like particles were labeled with anti–stage-specific embryonic antigen (SSEA)-5 monoclonal antibodies that bind a glycan highly and specifically expressed on the

surface of hPSCs. To assess killing efficiency and targeting specificity, single cultures of hPSCs and human dermal fibroblasts, as well as co-cultures of hPSCs with mouse embryonic fibroblasts were treated with SSEA-5-labeled Qβ(ZZ)@CD virus-like particles in the presence of the prodrug 5-fluorocytosine (5FC). After 24 hours, cytotoxicity in hPSC and human dermal fibroblast single cultures was quantified using a tetrazolium salt-based assay. The specificity of killing hPSCs in co-cultures was assessed via fluorescent microscopy using the dead cell-specific dye ethidium homodimer-1 and an antibody against the hPSC-specific surface marker Tra-1-60. In single cultures, near complete elimination of hPSCs was achieved, while virtually no cell death was observed in human dermal fibroblasts. In co-cultures, the dead cell-specific dye was brightly fluorescent specifically in hPSC colonies as demonstrated by co-localization with the Tra-1-60 antibody. No fluorescence was detected in surrounding differentiated cells, demonstrating both specificity and efficiency of virus-like particle-mediated cytotoxicity. The present study describes a novel implementation of a prodrug-mediated suicide system using antibody-labelled virus-like particles to efficiently and selectively eliminate undifferentiated hPSCs in vitro with virtually no cytotoxicity to differentiated cells. This technology is expected to be a useful tool in the prospective removal of undifferentiated cells in hPSC-based therapies without the disadvantages genetic transformation.

158. A Facile Nanotechnology Assay to Identify Stem Cell from Differentiated Cardiomyocytes
Qian, Ximei; Han, JJ; Wu, Qinling; Jha, Rajneesh; Yang, Z; Maher, Kevin O.; Nie, Shuming; Xu, Chunhui

Background: As the renewable source of all cell types in the human body, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) hold great promise for regenerative medicine and cell therapy. However, one major obstacle to the clinical application of these pluripotent stem cells (PSCs) is that these kinds of stem cells remaining with their differentiated derivatives pose cancer risk by forming teratomas after transplantation. Current cell-based assays including flow cytometry have limited detection sensitivity (>0.1% population). There is a great need to develop highly sensitive assay to meet the stringent safety standard for stem cell products (<0.001% population). Methods: Here we report a new method using surface-enhanced Raman spectroscopy (SERS) to detect residual undifferentiated cells in a highly sensitive and specific manner. A single-tube assay based on multiplexed bio-conjugated SERS nanoparticles has been developed. Two reporter molecules, BHQ, and BIDI, were used to encode gold nanoparticles to represent different signatures for pluripotency surface marker (TRA1-60) and stage-specific embryonic antigen-5 marker (SSEA-5), respectively. Results: The detection sensitivity of SSEA-5-, and TRA1-60- SERS nanoparticles has been evaluated by titrating 10-10,000 stem cells among 1 million fibroblast 3T3 cells. The linear correlation between SERS intensity and spiked tumor cell number indicates the limit of detection is in the range of 1-10 tumor cells per 1 million control cells for the ensemble measurement. The result shows that the SERS assay is able to detect stem cells at as low as 0.0001% whereas flow cytometry analysis carried out in parallel only shows a detection sensitivity of 0.1-1.5%. The assay specificity was also validated by comparing the SERS spectral intensity and of SSEA5-, and TRA1-60 expression levels on both embryonic stem cell H7 cell line and induced pluripotent stem cell (iPSCs) IMR90 cell line. Immuno-fluorescence imaging analysis shows agreement with SERS measurement. Conclusions: If the assay developed and validated by gold standard in vivo assay, it will hold great potential to serve as a fast, simple, inexpensive tool for stringent quality control of differentiated iPS cell therapy, which may exhibit excellent commercial entity to replace the expensive and time consuming in vivo test.

159. Indicators of RSV Disease Severity

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Respiratory syncytial virus (RSV) is the single most important cause of lower respiratory tract infections (LRTIs), pneumonia and bronchiolitis, in infants and young children worldwide. Although LRTIs are among the most important cause of disease and death in young children no RSV vaccine is yet available. The virus associated tissue damage and host inflammatory response contribute to the disease process. Presently, disease severity scores are based on clinical measures such as respiratory rate, O2 saturation, hospitalization, ICU admission, and mechanical ventilation. These measures are imprecise and only detect large differences in severity of disease and, consequently, require a large numbers of patients to be studied to detect a vaccine effect. Based on previous work, release of cytokines, chemokines and other biomarkers correlate with severity of the disease. Thus, various biomarkers have been associated with severity of disease but not comprehensively and simultaneously in the same study. In the present study, to develop disease severity index, we tested a variety of biomarkers including TNF-α, IL-17, IL-6, IL-8, IFN-γ, IL-10, IL-1RA, IL-4, IL-13, IL-5, IP-10, MCP-1, MIG, RANTES, VEGF, FGF-basic, EGF, HGF, PDGF-AB/BB, G-CSF, GM-CSF, IL-18, sICAM1, IL-2R, IL-12, IL-15, IFN-α, IL-2, IL-7, IL-1β, IL-1α, Eotaxin, MIP-1α, MIP-1β, Elastase 2, MPO and MUC5AC that have been previously associated with severity of RSV disease in previously collected and stored RSV positive nasal wash specimens (NWSs) from 63 children with mild (ambulatory, physician attended illness), 212 with moderate (hospitalized, no mechanical ventilation), 117 with severe (ICU admission and mechanical ventilation) disease and 118 RSV negative control group using multiplex luminex platform and Enzyme Immuno Assay (EIA). Our preliminary results show a link between certain cytokine/chemokine expression including IL-2, MUC5AC, Eotaxin and MPO and disease severity. The index developed in this study should provide the better quantitative measure of disease severity needed to decrease the cost of clinical trials and increase the speed of RSV vaccine development.

160. Regulation of HELLS as a Downstream Effector of Sonic Hedgehog Signaling in Cerebellar Development and Medulloblastoma

Robinson, M. Hope; Farrow, Hamza; Kenney, Anna M.

Aberrant hedgehog signaling has been implicated in a number of cancers, including medulloblastoma (MB), the most common malignant pediatric brain tumor. One of the molecular subgroups of MB is characterized by aberrant activity of the Sonic hedgehog (SHH) signaling pathway. Importantly, SHH mitogenic signaling can be modeled in vitro using primary cultures of cerebellar granule neuron precursors (CGNPs), which are dependent upon exogenous Shh for proliferation and are thought to be the cell of origin for the Shh subclass of medulloblastoma. In vivo these tumors can be modeled using mice bearing activating mutations in the Shh pathway. While some of the signaling alterations in SHH MB are due to gene mutations or amplifications, others may have their roots in epigenetics. Therefore, we examined levels of expression of several candidate epigenetic regulators in Shh-treated CGNPs. We identified lymphoid specific helicase (Hells) as a gene whose expression was markedly induced by Shh. Hells is a member of the SNF2 family of chromatin remodelers with multiple reported epigenetic functions in DNA methylation, histone acetylation and methylation, and chromatin remodeling in addition to roles in transcription activation and DNA repair. Overexpression of Hells has been observed in many cancers and

our preliminary analysis indicates overexpression in human medulloblastoma as well. We found that Hells expression is also increased in medulloblastomas from a mouse model for Shh MB. In addition to increased mRNA expression, we observed elevated levels of Hells protein in both our in vitro and in vivo models. Currently, we are determining the mechanism of Hells regulation by SHH and whether Hells is required for proliferation and survival. We have observed continued transcription of Hells with Shh stimulation even with inhibition of new protein synthesis, which may indicate Hells is an immediate-early gene. Additionally, to investigate SHH pathway regulation of Hells, GANT61, an inhibitor of Gli (Shh signaling effector) activity, was applied to CGNPs with the resulting dose dependent reduction of Hells expression suggesting Hells is a transcriptional target of Gli. We are also evaluating Hells intracellular localization and protein stability. To ascertain Hells involvement in proliferation and survival we are examining effects on these processes with overexpression and knockdown of Hells. In future studies we will test the hypothesis that Hells may have oncogenic functions, by transduction of mouse medullobastoma cells with Hells constructs and re-implanting into host pups, then assessing effects on tumor latency and growth.

161. Therapeutic Evaluation of MSC Delivery in a Small Animal Model for Juvenile Osteochondritis Dissecans

Salazar-Noratto, Giuliana E.; Stevens, Hazel Y.; De Nijs, Nica; Xu, Maojia; Willimon, S. Clifton; Barry, Frank; Guldberg, Robert E.

Juvenile Osteochondritis Dissecans (JOCD) of the knee is an increasingly common condition that predominantly affects adolescent and young adults, and progresses to early onset osteoarthritis. JOCD initially involves the formation of an avascular lesion in the subchondral bone with secondary effects in the overlaying articular cartilage. During late stages of this disorder, the lesion becomes unstable and separation of an osteochondral fragment (loose body) ensues. Previous research has been primarily limited to retrospective clinical studies, hampering the creation of novel therapeutics. Therefore, an animal model capable of evaluating putative therapeutic approaches is needed. In this study, we characterized a pre-clinical small animal rat model as a first step to tailoring diagnosis and fine tuning treatment of this musculoskeletal disorder, and to evaluate the efficacy of current clinical practice (microdrilling with/without delivery of bone marrow aspirate concentrate (BMAC)). In 12 Lewis rats of matched weight, we created an initial, localized necrotic lesion near the subchondral bone with a cryogenic insult. Histology confirmed that there was osteonecrosis in the surrounding area and the overlaying cartilage was compromised, as marked by a decrease in proteoglycan content. However, further optimization needs to be performed in order to localize the lesion. In parallel, JOCD patient-specific mesenchymal stem cells (MSCs) are being generated from skin biopsies via induced pluripotent stem cell technology. Patient- and control-specific MSCs will be used in vitro to identify potential JOCD phenotype deficiencies in cell function (osteogenic and chondrogenic differentiation) and, in the small animal model, to evaluate deficiencies in cell delivery in the microdrilling model. Overall, establishing a small animal model of OCD with the ability to emulate the progression of this disorder could serve as a platform to explore novel treatment procedures, as well as to optimize current clinical approaches.

162. Adaptive Behavior Profiles in Girls with Autism: A Comparison to Previously Published Profiles in Boys

Saulnier, Celine; Moriuchi, Jennifer; Klin, Ami

Background: The vast majority of research on adaptive behavior profiles in individuals with autism spectrum disorders (ASD) has been conducted on boys. In boys with ASD without cognitive impairment, adaptive skills tend to fall substantially below both IQ and age, with age being negatively correlated with adaptive behavior, suggesting a widening gap with age (Klin, Saulnier, Sparrow et al., 2007; Kanne et al., 2010). Objectives: This study compares a sample of girls with ASD with a sample of previously published boys of the same age range and studied at the same institution (Klin et al., 2007). In the Klin study, 84 boys 8 to18 years (Mean=12.4) of average intelligence (FSIQ=99.8) exhibited significant adaptive deficits, with their Socialization Standard Scores on the Vineland falling more than 3 standard deviations below chronological and mental age (Mean=52.0). Age was negatively correlated with adaptive functioning, with older boys exhibiting a greater gap between cognition and adaptive behavior than younger boys. The current study aims to examine if girls with ASD exhibit similar profiles. Methods: Participants included 48 girls with ASD that received diagnostic evaluations through the Yale Developmental Disabilities Clinic. The sample was restricted to girls between the ages of 8 and18 years to match the Klin et al. sample (Mean Age=11.47; SD=2.63). IQ measures included the Differential Ability Scales, Second Edition and a combination of the Wechsler Scales for children for adults. The Vineland Adaptive Behavior Scales, Expanded Form was used to assess adaptive behavior. Results: Results revealed significant delays in all adaptive areas with the following Vineland Mean Standard Scores: Communication=64.77 (SD=22.59); Daily Living Skills=53.02 (SD=21.66); and Socialization=56.65 (SD=15.0). Pearson correlations indicated that Nonverbal IQ was related to more areas of adaptive functioning (Comm r=.56, p<.01; DLS r=.48, p<.01; Soc r=.34, p<.05) than Verbal IQ (Comm r=.62, p<.01). Age was negatively correlated with adaptive Socialization skills (r= -0.31, p<.05), consistent with results in boys. Conclusions: Results highlight the similarities in adaptive behavior profiles in girls compared to well-established profiles in boys. Though current studies are questioning if diagnostic profiles of girls could be qualitatively different than those in boys, our findings suggest that perhaps differences in symptomatology could be independent from more consistent cognitive and adaptive profiles. The substantial gap between cognition and adaptive functioning is similarly striking, as is the significant correlation between age and socialization skills suggesting that this gap widens with age. Implications for informing intervention will be discussed.

163. A Consecutive Case Review of Token Systems Used to Reduce Socially-Maintained Challenging Behavior in Individuals with Intellectual and Developmental Delays

Scheithauer, Mindy; Mauzy, Courtney; Cariveau, Tom; Call, Nathan; Ormand, Hailey; Clark, Seth

Objectives: The current paper describes the use of token systems in a behavioral day-treatment unit for severe challenging behavior using a consecutive case review spanning three years. Suggestions for the implementation of token systems within clinical settings are offered and areas for future research are outlined. Methods: Experimenters evaluated 96 cases, 24 of which implemented some token system as a component of the treatment package. Aspects of each token system (including schedules of token delivery and exchange; inclusion of token training and response cost; and types of back-up reinforcers delivered) and participant information (including age, race, diagnosis by history, topographies of challenging behavior, and function of challenging behavior) were coded. Results: Token systems were

most frequently employed during differential reinforcement for alternative behavior (DRA, most commonly for compliance) or differential reinforcement of other behavior (DRO). Tokens and were most frequently used in a demand context. Several commonalities were identified between cases (e.g., restriction of back-up reinforcers between token exchanges, initial dense schedules of reinforcement). Compared to past reviews, this sample had an over-representation of individuals with challenging behavior maintained by escape, multiply maintained challenging behavior, and individuals for which the function was not identified in a functional analysis. Treatment packages including token systems resulted in a reduction in challenging behavior for 91.67% of participants, with 70.83% exhibiting at least an 80% reduction. Conclusion: Our findings suggest that token systems may be a beneficial component of treatment plans used to address challenging behavior. Some common components should likely be included in all token systems and specific client variables may guide decision related to more idiosyncratic components.

164. DNA Methylation Profiling for Validation of Pediatric Brain Tumor Neurosphere Cultures

Schniederjan, Matthew; Bowman, Chistopher; Karajannis, Matthias; Serrano, Jonathan; Snuderl, Matija; Moser, Cathey; Rogers, Beverly; Macdonald, Tobey

Background: In vitro tissue culture of human tumor cells has been an important aid to the study of cancer, yet there are major difficulties in generalizing findings from such material to in vivo models. A primary concern is the effect of culture conditions on the behavior and biology of tumor cells, i.e. whether the cells continue to represent the tumor as it existed in the patient. DNA methylation profiling is a powerful and robust method to assess a genome-wide methylation pattern, providing a sort of epigenetic “fingerprint” for a given tissue and could be a way to test the similarity of cultured cells to original tumors. Methods: To compare cultured cells and original tumor, we tested cryopreserved viable tumor tissue, paraffin embedded tumor, and cultured neurospheres from five malignant pediatric brain tumors, including four medulloblastomas and one glioblastoma. DNA was extracted from all samples and run on an Infinium Methylation EPIC BeadChip microarray and scanned on the Illumina iScan according to the manufacturer’s instructions. Data analysis was performed using RnBead version 1.3.6. Results: Two of the neurosphere cultures displayed very similar profiles to their source tumor tissue and paraffin material, clustering tightly with them, whereas two other neurosphere cultures showed marked divergence in methylation pattern from the source material. Scanning failure for one of the medulloblastoma neurosphere specimens prevented its further comparison. Each pair of the paraffinized and frozen fresh tumor specimens showed high degrees of overlap, indicating no significant difference in methylation pattern for those two conditions. Conclusions: DNA methylation profiling showed different degrees of clustering for different neurosphere cultures. Two of the cultures had profiles that closely matched those of their original source tissue, and two had profiles that were very different. It is unclear what the underlying cause of these differences is and what these differences mean about the biology of those cultures. Based on these observations, DNA methylation profiling is a promising tool for validating the nature of human, brain-tumor-derived neurosphere cultures.

165. Guide to Goals Program: mHealth Application to Implement Evidence Based Practice in Pediatric Type 1 Diabetes Management

Shankar, Prabhu; Owens, Shane; West, Leanne; Muir, Andrew

Background: The goal of this pilot is to promote implementation of American Diabetic Association (ADA) standards of care (SOC) for children with type 1 diabetes (T1D) using point-of-care informatics tools. T1D affects 2-3/1,000 Americans under age 20, and the prevalence is rising. Implementation of best practices substantially reduces mortality and morbidity, yet 40-80% of children cannot maintain ideal blood glucose control. The Chronic Care Model (CCM) addresses this shortfall by assembling a multi-disciplinary healthcare team to inform families, allowing them to establish evidence-based goals for their own treatment. The ADA's SOC provide a sound basis for goal-oriented therapy; but, their systematic implementation is cumbersome. We propose a novel electronic guide to pediatric T1D care to: 1) promote goal setting by patients and behavior change towards consistent education by providers and 2) comprehensively monitor 17 evidence-based SOC for pediatric T1D. Method: Guide to Goals (GTG) is a mobile app and an expert alert system (AS), designed for pediatrics. It employs a tablet-based front-end, a backend relational database, and an alert system based on ADA guidelines. The tablet is used for data entry, data retrieval (e.g., questionnaires and laboratory results), and alert notifications. After assigning a secure login upon patient arrival, patients/caretakers will use the tablet to complete questionnaires, historical/clinical updates and lab values. Captured data is stored in the backend database, but plans include future EHR integration. By applying the rules engine to the database, the AS will trigger real-time alerts and reports about each patient's progress, their goals and morbidity and mortality risks. Providers can generate specific alert-based management and educational instructions to improve patient adherence to the ADA standards. Results and future steps: The application has been developed and usability testing is underway. Focus group meetings are planned with end users to gather suggestions for improvement and clinical trials will be launched in 3 pediatric endocrinology academic/private practices in Atlanta. Trial outcomes will measure the app's ability to:-Promote behavior change towards more consistent application of the Chronic Care Model (CCM) by providers and by patients-Monitor evidence-based standards of care-Encourage patient-directed goal-driven therapy-Maximize and standardize patient education-Optimize intra-team communication and -Provide reliable data for outcomes analysis. GTG will provide the basis for designing a measure of the quality of pediatric T1D care. To our knowledge, there are no validated pediatric-specific applications in the literature.

166. Combining Informatics Resources and Research Methods for Predictive Analytics of Premature Infants with Pulmonary Hypertension

Shankar, Prabhu; Travers, Curtis; Vyas-Read, Shilpa

Objective: To mine, integrate, aggregate and analyze data about patients with risk for Bronchopulmonary Dysplasia (BPD). Aims were to determine factors associated with Pulmonary Hypertension (PH) in the BPD population and develop a predictive model for PH>30 days of life. Background: Approximately 45% of premature infants with gestational age <32 weeks and very low birth weight of <1500 grams need respiratory support with oxygen at 28 days of life, the clinical case definition of BPD. Infants with moderate-to-severe BPD continue to require oxygen/respiratory support at 36 weeks corrected gestational age (actual age in weeks - weeks premature = corrected age). About 8-23% of premature infants develop PH, and up to 50% of infants with PH die by 3 years. PH screening is recommended in

premature infants but evidence is scare about which patients warrant screening. We aimed at delineating risk factors for PH in the mined BPD patients. Methods: Children's Healthcare of Atlanta based Population Discovery, Clarity and Cardiac Imaging databases were queried from 2010 - 2015 for billing codes (for BPD), Infants with weight <1500 grams, and a gestational age <32 weeks at birth and PH in echocardiography. Congenitally anomalous patients were excluded. Further query was performed for respiratory support, including various modalities of oxygen therapy. Cohort of patients with echocardiographic evidence of PH at any point, and after >30 days, were used as primary outcomes and predictive modeling. With SAS 9.4 descriptive statistics, univariate and multivariate logistic regressions were conducted. Results: 407 patients had a BPD diagnosis code of APR-DRG 132 or ICD 770.7. Manual curation with aggregated data identified 626 patients with possible BPD. 559 infants were included in the overall study, and 92 (16.5%) had PH. Black race (1.79, 1.02-3.14), atrial septal defect (ASD, 2.72, 1.45-5.11) and patent ductus arteriosus (2.04, 1.19-3.49) increased the odds of PH in multivariable analyses, whereas treatment with caffeine decreased the odds (0.49, 0.29 -0.84). 321 infants were in the PH>30 days of life group and a model of birth (birth weight, Apgar 1 minute, Black Race) and early neonatal variables (caffeine, ASD), with positive-pressure ventilation controlled was determined. The ROC showed an area of 0.766, corresponding to a validated sensitivity 80%, specificity 44%. Conclusion: A combination of informatics resources need to be used for cohort definition to fill the missing and inaccurate billing codes data. Phenotype characterization and cohort identification algorithms for BPD patients need to consider the above mentioned factors.

167. Trends in Neonatal Surgical Outcomes in Children's Versus Non-Children's Hospitals

Short, Heather; Savinkina, Alexandra; Raval, Mehul

Purpose: Newborns undergoing surgery represent one of the most fragile patient populations and require specialized care. Our purpose was to examine trends in neonatal surgical outcomes between children's and non-children's hospitals (CH and NCH). Methods: A cross-sectional, retrospective review of the 2000, 2003, 2006, 2009 and 2012 Kid's Inpatient Database (KID) was performed to identify all neonatal surgical cases of necrotizing enterocolitis (NEC), patent ductus arteriosis (PDA), esophageal atresia/tracheoesophageal fistula (EA/TEF), congenital diaphragmatic hernia (CDH), and gastroschisis/omphalocele (GAS/OMP). Mortality rates, length of stay (LOS) and hospital costs at CH and NCH were compared. Results: We identified 48,149 patients who underwent a surgical procedure to correct one of the diagnoses of interest during the neonatal period. During the 12-year study period the incidence of all diagnoses increased. The majority of patients (73%) were treated at NCH, however the proportion of children treated at CH increased 12%, from 18.4% to 30.3%, during the study period. Overall mortality decreased from 14.9% in 2000 to 12.6% in 2012. This improvement is largely due to an improvement in mortality at CH from 17.2% in 2000 to 10.9% in 2012, while mortality in NCH remained stable at about 14%. Mortality was consistently lower at CH than NCH for 4 of 5 diagnoses, excluding NEC for all study years and CDH in 2006. From 2000 to 2012, overall mean LOS increased from 44 to 57 days and this trend was similar in CH and NCH. However, when individual diagnoses were examined LOS was longer in CH than NCH every year for all diagnoses except PDA. After adjustment for inflation, there was a two-fold increase in cost per day for all diagnoses from $5,015/day in 2000 to $10,508 in 2012. Cost per day was higher for each diagnosis at CH compared to NCH in each year. In 2000 these neonatal conditions cost $1.2 billion (22% at CH) and in 2012 cost increased to $7.6 billion (35% at CH). Conclusions: Mortality among neonates undergoing surgery is improving at CH and is stable at NCH.

However, LOS and costs are consistently higher at CH than NCH. In order to optimize outcomes and contain costs for these fragile patients the observed trends warrant further investigation.

168. Association Between Operative Time and Outcomes in Children's Surgery
Short, Heather; Fevrier, Helene; Meisel, Jonathan; Santore, Matthew; Heiss, Kurt; Wulkan, Mark; Raval, Mehul

Introduction: Prolonged operative time (OT) is often considered in risk-adjusted models as a reflection of procedural complexity and has been shown to be associated with poor outcomes. Our objective was to explore the association between prolonged OT and postoperative complications in children's surgery. Methods: All 182,836 cases from the 2012-2014 American College of Surgeons National Surgical Quality Improvement Program-Pediatric (NSQIP-P) were organized into 33 procedure groups. OT for each group was analyzed by quartile, and regression models were used to determine the relationship between prolonged OT and overall, minor, and major postoperative complications. Minor complications were defined as pneumonia, any wound infection, shock, or reintubation, while major complications were defined as renal failure, cardiac arrest requiring CPR, sepsis, pulmonary embolism, ventilation after 24 hours, cerebrovascular event, readmission, reoperation, or mortality. Results: Large variations in OT existed for both short (congenital abdominal wall defects [median: 33 min, Interquartile Range (IQR): 48, 76]) and long procedures (spine surgery arthrodesis [median: 253 min, IQR: 175, 429]). Cases in the longest quartile for OT had twice the odds of having postoperative complications after adjusting for age, sex and BMI (OR 1.85; 95%CI 1.78-1.91). Procedure-specific prolonged OT was associated with postoperative complications for the majority (85%) of procedural groupings. Prolonged OT was highly associated with minor complications in gynecologic (OR 4.17; 95% CI 2.19-7.96), urologic (OR 2.88; 95% CI 2.40-3.44), and appendix procedures (OR 2.88; 2.49-3.34). There were increased odds of major complications with prolonged OT in foregut (OR 6.56; 95% 4.99-8.64), gynecologic (OR 3.07; 95% CI 1.84-5.13), and spine arthrodesis procedures (OR 2.99; 95% 2.57-3.28). Some procedure groups demonstrated an association between prolonged OT and major complications but not with minor complications (i.e. airway, colorectal, and facial plastics). Very few (12%) procedural groups failed to demonstrate an association between OT and outcomes (i.e. neurosurgical shunts, cholecystectomy, and splenectomy). Conclusions: Prolonged OT is associated with increased risk of postoperative complications across a broad spectrum of children's surgical procedures. Modifiable factors contributing to prolonged OT merit further investigation and may serve as a target for future quality improvement efforts.

169. Implementation of a Pediatric Specific Enhanced Recovery Protocol
Short, Heather; Travers, Curtis; Burch, Katelyn; Heiss, Kurt; Raval, Mehul

Introduction: Enhanced recovery after surgery (ERAS) protocols have been shown to decrease hospital length of stay (LOS) and complications in diverse adult surgical populations. Our purpose was to compare outcomes before and after the implementation of a pediatric specific enhanced recovery protocol (ERP) in children undergoing elective colorectal surgery. Methods: A multi-disciplinary approach was used to develop a pediatric specific ERP, and we began implementing it at our institution in January 2015. The protocol included the following 17 elements: preadmission counseling, no prolonged preoperative fasting,

standard bowel preparation, administration of a preoperative loading dose of non-narcotic pain medication, preoperative antibiotic prophylaxis, sequential compression device placement, removal of foley or gastric tube at the end of the case, regional anesthesia, minimization of intraoperative narcotic administration, maintenance of intraoperative normothermia, minimization of intraoperative fluid administration to 3-4 mL/kg/hr, early postoperative mobilization, aggressive pulmonary toilet, early postoperative oral nutrition, maintenance of near zero fluid balance postoperatively, minimization of postoperative narcotic administration, and prevention of postoperative nausea and vomiting. We retrospectively reviewed 53 patients that underwent an elective colorectal procedure between January 2012 and December 2015 and tracked which ERAS elements each patient received. Outcomes of interest included median LOS, complication rate and 30-day readmission rate. Results: There were 43 patients (81%) in the pre-ERP period between January 2012 and December 2014 and 10 patients (19%) in the post-ERP period between January 2015 and December 2015. The median number of ERAS elements received per patient in 2012, 2013, and 2014 were 5, 5.5, and 6 respectively. In the post-ERP period, the number of ERAS elements received per patient increased to 10. During the study period, the median LOS decreased from 5 days in 2012 to 2 days in the post-ERP period. The complication rate (23% vs. 20%) and the 30-day readmission rate (19% vs. 20%) remained stable in the pre- and post-ERP periods. Additionally, the median time to regular diet, median volume of intraoperative and postoperative narcotics, and median volume of intraoperative fluids all decreased in the post-ERP period. Conclusions: These preliminary results suggest that implementation of a pediatric specific ERP in children undergoing colorectal surgery is feasible, safe and may lead to shorter LOS. Further investigation is warranted to justify the standard use of an ERP in all children undergoing colorectal surgery.

170. Cryopreserved Human Pluripotent Stem Cell-Derived Cardiomyocytes are Molecularly and Functionally Comparable to their Continuously Cultured Counterparts

Singh, Monalisa; Wu, Qingling; Preininger, Marcela K.; Jha, Rajneesh; Li, Jun; Cho, Hee Cheol; Wagner, Mary B.; Xu, Chunhui

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have been routinely studied in academic, clinical and industrial research fronts and have wide spread implications in cell therapy, disease modelling, and drug discovery. Employing freshly derived hPSC-CMs for these applications could be labor- and time-intensive due to long term cultures and in depth characterization prior to their use. Cryopreserved hPSC-CMs could overcome these limitations and provide a convenient, cost effective and efficient cell source for such downstream applications. However, structural and molecular effects of cryopreservation of hPSC-CMs remain unexplored and whether freeze-thaw alters functional efficiency is largely undetermined. We sought to answer these questions by a side-by-side comparison of cryopreserved and continuously cultured hPSC-CMs. Using tissue engineering techniques; we constructed 3D cardiac spheres upon thawing and successfully recovered up to 70% of cryopreserved cells. We further demonstrated that these recovered CMs exhibit similar levels of CM-associated markers, intracellular calcium handling properties, pharmacological responses and ability to electrically couple with rat myocytes in co-culture as their continuously cultured counterparts. These observations suggest that cryopreservation of hPSC-CMs is a feasible approach that can replace fresh derivation of hPSC-CMs in various applications, circumventing the time-consuming and labor-intensive maintenance of continuous cultures.

171. Orienting Response to Social Versus Physical Audiovisual Synchrony Does Not Differ in Toddlers with ASD

Sifre, Robin; Klin, Ami; Jones, Warren; Shultz, Sarah

Background: Preferential orienting to biological motion, a critical skill for early social development, is disrupted in toddlers with autism. Instead of orienting to biological motion, ASD toddlers attend to audio-visual synchrony (AVS), suggesting that their attention is guided by physical rather than social contingencies. Despite this apparent dichotomy, it should be noted that while AVS is indeed characterized by physical contingencies (the simultaneous presentation of light and sound), high AVS signals can also contain critical social information (i.e., co-speech gestures). This raises important questions about the adaptive value of orienting to AVS in typical development, and whether attention to AVS observed in ASD toddlers is truly driven by preferences for physical contingencies.
Objectives: Investigate whether TD and ASD toddlers attend differently to high-AVS signals generated by SOCIALLY-MEANINGFUL versus PHYSICAL contingencies. Methods: 58 toddlers (21 ASD, 37 TD) were shown point-light biological motion animations. An upright animation was presented on one half of the screen with the soundtrack of the actor's vocalizations. On the other screen half, the inverted version of the animation played in reverse order. Levels of AVS for each figure were quantified by measuring synchronous change in motion and sound at each frame. High-AVS moments were defined as frames with AVS values exceeding the 90th percentile threshold. High-AVS moments generated by the UPRIGHT FIGURE were classified as SOCIALLY MEANINGFUL, as they were generated by the movements and vocalizations of a biological figure. High-AVS moments generated by the INVERTED FIGURE were classified as PHYSICAL, as they were generated by the coincident alignment of disparate signals of light and sound. The mean probability of looking at the upright/inverted figure, was assessed 1500ms before and after a high-AVS event. If toddlers distinguish between socially-meaningful and physical AVS, then they should orient differently to AVS generated by the upright versus inverted figure. Results: While ASD toddlers showed remarkably similar orienting to AVS on the upright and inverted side at all time-points (ICC=0.89, p<.000001), this relationship was weaker for TD toddlers (ICC=0.14, p<.000001). TD toddlers showed anticipatory looking towards the upright figure BEFORE socially meaningful high-AVS events, after which they demonstrated sustained looking. Anticipatory and sustained looking were not observed for physical high-AVS events. Conclusions: While TD toddlers differentially orient to socially-meaningful and physical AVS, ASD toddlers do not. These results expand on findings that ASD toddlers preferentially attend to physical contingencies, while highlighting the possibly adaptive value of orienting to socially-meaningful AVS.

172. Differences in Patterns of Engagement with Scene Content Between School-Age Children with and without Autism Spectrum Disorder

Stallworthy, Isabella; Coben, Ella; Yurkovic, Julia; Jones, Warren; Klin, Ami; Shultz, Sarah

Atypical perceptions of the social world are a hallmark feature of Autism Spectrum Disorder (ASD). When viewing social scenes, individuals with ASD spend less time looking at the eyes of others and more time looking at less socially relevant features, such as objects (Rice et al., 2012). For greater insight into the subjective experience of individuals with ASD, this study examines not only where children with ASD look when viewing social scenes, but also how engaged they are with scene content. Probabilistically, people are least likely to blink when looking at what they perceive to be most important and most likely to blink

during moments perceived to be least important. Thus, by measuring patterns of visual fixation and blinking we can examine where children with ASD look when they are highly engaged. Objectives: To investigate: (1) where children with and without ASD look when viewing naturalistic social scenes; and (2) whether looking patterns are modulated by engagement with scene content. Eye-tracking data were collected while 109 children with ASD (mean age=10.32 years) and 40 age- and IQ-matched TD children (mean age=10 years) watched age-appropriate movies. Permutation testing identified periods of statistically significant blink inhibition (when children were highly engaged) and statistically significant increased blinking (when children were less engaged) for each group separately. Percentage of visual fixation time on eyes, mouth, body, and object regions were calculated for each child over: (1) the entire viewing session; (2) periods of high engagement; and (3) periods of less engagement. Multivariate ANOVAs showed that, over the entire viewing session, TD viewers fixated more on eyes and mouths compared to children with ASD, who instead looked more at objects and bodies (all p's<0.0001). These group differences were also observed during periods of high engagement (all p's< 0.05). Finally, there was a significant interaction between diagnosis and level of engagement, with TD viewers looking more at mouths when highly engaged compared to when they were less engaged (p<0.05). Results show that children with ASD and their TD peers perceive social stimuli in markedly different ways. TD viewers attend more to faces, while viewers with ASD attend more to objects. Critically, these differences become even more pronounced during periods when viewers were highly engaged with the stimuli. Ongoing analyses, examining between-group differences in the timing of when children are engaged and with what type of content, will further elucidate the subjective experience of individuals with ASD.

173. Production, Characterization and Immunogenicity of Candidate Ebolavirus VLP Based Vaccines

Singh, Karnail; Chen, Xuemin; Wang, Jaang Jiun; Blinder, Yelena; Spearman, Paul

Ebolaviruses are highly infectious pathogens that cause Ebola Viral Disease resulting in high rates of mortality. Knowledge of immune responses to these viruses is limited partly because of lack of infrastructure needed to perform research with these viruses. Currently there is no licensed vaccine in use that affords protection against these deadly viruses. Expression of Ebola virus antigens in non-infectious virus-like particles (VLPs) provides a means of presenting antigens in native conformation in a safe and practical format. Here we report the development of two stable cell lines secreting VLPs that express high levels of Ebola Zaire glycoprotein (EBOVGP) on either Ebola matrix protein VP40 or HIV Gag core. Negative-staining electron microscopic analysis revealed that VLPs with HIV Gag core were spherical and densely covered with GP on their surface while those with Ebola virus VP40 core were filamentous and also demonstrated a high level of Ebola GP incorporation. Immunization of rabbits with either EBOVGP+ VP40 or EBOVGP+ Gag VLPs produced high-titred antibodies that mediated ADCC dependent killing of the target cells expressing surface EBOVGP. These antibodies neutralized not only the Zaire but also the Sudan, Bundibugyo and Tai Forest species of Ebolavirus. These findings highlight the potential of VLP based candidate Ebola vaccines to elicit strong anti-Ebola immune responses.

174. Using Serial Exercise Testing to Guide Treatment of Pediatric Patients with Tetralogy of Fallot
Stark, Megan; Friedman, Heather; Fischbach, Peter

Background: There is limited research on the usefulness of exercise stress testing data in the pediatric population with tetralogy of Fallot (TOF); however there is growing evidence in the adult population that serial exercise data can aid in the timing of pulmonary valve replacement surgery (PVR) and risk stratifying of exercise induced arrhythmias. There is a patient dependent, time-sensitive point at which the decrease in cardiac function due to pulmonary valve stenosis cannot be reversed. While serial exercise testing could identify that time point for individual patients, there are currently no accepted protocols for exercise testing in TOF patients. The purpose of this study was to examine ordering practices and to investigate the need for an established protocol at Children's Healthcare of Atlanta (CHOA). Methods: During the study period, 30 patients with TOF were identified by searching the exercise lab database. Theses individuals performed maximal exercise tests on either a treadmill using a Bruce protocol or cycle ergometer using a James protocol in the Stress Lab at CHOA. Demographic and testing data were extracted from their medical records. These data were retrospectively analyzed. Results: The mean age of the patients' at the time of their test was 13.5 years. All patients reached a maximum effort (mean RER ≥ 1.10). Percent predicted VO2max for this set of TOF patients ranged from 42-139% predicted. The average VO2max %predicted for this population was 83.4%. Serial data for four patients was available. Discussion: We conclude that there is a need for uniform standards in the pediatric population using exercise stress testing data to assist in timing of pulmonary valve replacement. One patient who had the lowest predicted VO2max before PVR had worsening function after PVR. This suggests surgery was delayed too long. One with deteriorating performance on exercise testing underwent PVR and demonstrated improved VO2max post operatively suggesting surgery occurred prior to irreversible damage. Further research would assist in determining the point at which a decrease in cardiac function can be reversed. At our facility, we are in the process of implementing a protocol to guide TOF care. It is necessary to establish a set of normative data for absolute comparison and perform serial exercise testing for relative comparison of VO2 max. Performing serial tests will allow intra-patient data comparison, aiding cardiologists in determining the appropriate timing of PVR.

175. S. aureus Sphingomyelinase is a State-dependent Inhibitor of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
Stauffer, Brandon; Cui, Guiying; Infield, Danny; McCarty, Nael

Background: Cystic Fibrosis (CF) is a devastating congenital disease that afflicts ~70,000 people worldwide and results from loss-of-function mutations in the epithelial chloride channel Cystic Fibrosis Transmembrane conductance regulator (CFTR). CF patients commonly present with persistent pulmonary bacterial infections that correspond with a worsening of clinical prognosis. Sphingomyelinase (SMase), an enzyme that cleaves the membrane lipid sphingomyelin into phosphorylcholine and ceramide, is a secreted bacterial virulence factor that has been shown to inhibit CFTR chloride channel function and may contribute to the observed exacerbation of disease by dehydrating airways and facilitating bronchial plugging. The goal of this study is to determine the mechanism by which SMase inhibits CFTR channel activity. Results: Our data suggests that isolates of S. aureus collected from the airway of CF patients avidly secrete SMase in vitro. We found that S. aureus isolates caused hot/cold hemolysis when grown on blood agar plates, and conditioned mediate from liquid cultures of the same

isolates showed significant SMase activity in a fluorometric, SMase-specific assay. Additionally, we show that inhibition of CFTR channel activity by S. aureus SMase occurs via a novel, state-dependent process. Specifically, inhibition occurs at the plasma membrane and does not rely on the regulatory (R) domain. Patch clamp experiments suggest that the effect relies on an intracellular factor because channels in excised patches are insensitive to inhibition. Finally, experiments performed with mutant variants of CFTR showed that channels with increased activity have a decreased sensitivity to inhibition by SMase, whereas mutations that decrease channel activity increase sensitivity to inhibition. Conclusion: Taken together, these data suggest that S. aureus isolated from CF patients avidly secrete SMase, a virulence factor that acts as a state-dependent CFTR inhibitor in vitro.

176. Aggregation of Child Cardiac Progenitor Cells into 3D Spheres Improves Endothelial Lineage Commitment

Trac, David; Xu, Chunhui; Davis, Michael

Congenital heart disease is rarely cured by surgery and can lead to life-threatening, intractable right ventricular heart failure (HF). In particular, children with hypoplastic left heart syndrome have a 10 year transplant-free survival rate of 50-75% despite palliative surgical repair. Currently, no effective stem-cell based treatments are available for pediatric HF. Recent stem-cell based clinical trials have been limited by poor differentiation rates and low cell retention. Additionally, we have shown that human cardiac progenitor cells (hCPCs) have reduced regenerative potential as they age, starting as early as 1 year old. We propose the aggregation of CPCs into scaffold-free spheres to improve the differentiation of child CPCs into mature cardiac phenotypes by enhancing intercellular Notch signaling. Notch signaling activity has been implicated in the regulation of CPC fate decisions and prior research in our lab has shown that intramyocardial delivery of Notch-ligand containing hydrogels improves cardiac function. Child CPC spheres were produced at a size of 1500 cells per sphere using a microwell array and cultured in suspension. Using immunohistochemistry, we showed that aggregation of CPCs increased Notch1 expression compared to parallel monolayer cultures. This effect is not limited to CPCs and was recapitulated in spheres of Chinese hamster ovarian cells transfected with Notch1-YFP. Additionally, Notch signaling pathway gene array data showed increased expression of the Notch-cleaving metalloprotease ADAM10 (3.6-fold) and Notch ligand DLL1 (25.0-fold) in CPC spheres by 3 days in culture compared to monolayer cultures. By 14 days in culture, we showed that aggregation of CPCs robustly increases the expression of the GATA4, a cardiac transcription factor associated with angiogenesis, and VEGFR1, an early marker of endothelial lineage commitment. Based on our results, we hypothesize that aggregation of CPCs into spheroids increases endothelial differentiation via a Notch-dependent mechanism. Transplantation of CPC spheres may improve cardiac function in vivo compared to transplantation of single CPCs. The results from our project will facilitate the development of autologous stem-cell based therapies for pediatric HF.

177. Jitter and Blocking Pre- and Post-Treatment in Pediatric Generalized Myasthenia Gravis

Verma, Sumit; Collop, Marie; Lin, Jenny

Background: Stimulated jitter analysis (stim-ja) of the orbicularis oculi muscle using concentric needle electrode examination is a sensitive and well-tolerated technique to diagnose MG in children1. However,

serial stim-ja studies to evaluate MG disease course (pre- and post-treatment) is not done in children 2, 3. In absence of validated pediatric MG scales an objective electrophysiological test correlating with clinical improvement would be helpful. Objective: To measure jitter and blocking pre- and post-treatment in pediatric MG. Design/methods: Five girls (age range 4-16 years) with seropositive generalized MG followed in the pediatric MG clinic at a tertiary care Children's hospital over the period of 2014-2016 were studied. Three subjects were newly diagnosed and the other two were referred to MG clinic with poorly controlled symptoms. Serial stim-ja (jitter/ blocking) studies and clinical exam were performed. Subjects were treatment (pyridostigmine 5/5, corticosteroids 5/5, azathioprine 1/5, IVIG 3/5, thymectomy 1/5, PLEX1/5) with goal to achieve complete remission. Results: Twelve stim-ja studies were performed without complications or need for sedation. Two hundred seven apparent single fiber action potentials (ASFAP's) were recorded. Mean interval between two stim-ja studies on study cohort was 13.6 ± 5.3 months. Jitter measurement (mean MCD pre-treatment 82±37 µs, post-treatment 34±7 µs, p≤0.03) and % ASFAP's with blocking (pre-treatment 55±33 % and post-treatment 0%, p≤0.01) showed statistically significant improvement. Follow-up clinical examination recorded interval improvement in ptosis (80%), ophthalmoplegia (100%), facial (70%), bulbar/ respiratory (100%) and proximal muscle strength (75%). Discussion: Statistically significant improvement in jitter and blocking correlated with clinical improvement in treated cases of pediatric MG. Study limitations were that the electromyographer was not blinded (subject clinical course) and relative small study numbers. Stim-ja can be used as an objective measure of clinical improvement in pediatric MG. Reference 1.Verma S, Lin J. Stimulated jitter analysis for the evaluation of neuromuscular junction disorders in children. Muscle Nerve 2016; 53 (3): 471-472. 2.Massey JM, Sanders DB, Saperstein DS et al. Comparison of single fiber EMG studies to outcome measures in a controlled study of mycophenolate mofetil in generalized MG. Annals of NY Acad Sciences 2008; 1132; 377 (abstract). 3. Zinman L, Baryshnik D, Bril V. Surrogate therapeutic outcome measures in patients with myasthenia gravis. Muscle Nerve 2008; 37: 172-176.

178. Post-translational Modification of I2PP2A by PI3K Pathway in Shh Medulloblastoma

Wei, Yun; Kenney, Anna M.

Medulloblastoma is the most common solid brain malignancy of childhood. Approximately 30% of human medulloblastomas show aberrant Sonic Hedgehog (Shh) pathway activity. To study Shh-driven mitogenic and oncogenic signaling pathways, we use the NeuroD2-SmoA1 mouse model to investigate how downstream effectors of Shh signaling regulate cell proliferation in medulloblastoma development. I have been focusing on the serine-threonine protein phosphatase 2A, deregulation of which has been reported in many tumors. PP2A can dephosphorylate and inactivate critical oncogenic kinases such as S6K and Akt, thus it is considered to be a tumor suppressor. Three endogenous inhibitors of PP2A are reported to be tumor-promoting, designated as CIP2A (cancerous inhibitor of PP2A), I1PP2A and I2PP2A (inhibitor 1 and inhibitor 2 of PP2A, respectively). I found that protein level of I2PP2A but not I1PP2A or CIP2A is markedly elevated in mouse medulloblastomas compared with neighboring normal cerebellum. The upregulated I2PP2A has been reported in other solid tumors which promotes tumor growth and survival by inhibiting several tumor suppressors such as PP2A, NME1 (non-metastasis marker 1). Also, researchers found dysregulation of I2PP2A post-translational modification play roles in hyperphosphorylation of Tau in Alzheimer's disease, a neurodegenerative disease in the brain. Findings about I2PP2A in other diseases make it a potential therapy target in Shh medulloblastoma which needs more investigation. The observations of upregulated protein but not mRNA levels of I2PP2A indicated that

the post-translational modification of this protein may help stabilize this protein in Shh medulloblastoma. PI3K (Phosphoinositide 3-kinase) pathway plays significant roles in cancer cell survival and radiation resistance of Shh medulloblastomas and has been shown to regulate I2PP2A by post-translational modification in other organs. Whether PI3K is the signaling cascade that regulates I2PP2A is still a question. My research will focus on two important topics, whether I2PP2A plays roles in promoting cancer cell survival and whether PI3K pathway is the upstream signal that regulates I2PP2A in Shh medulloblastoma. Ultimately I hope to extrapolate this research into a novel therapy design targeting PI3K:I2PP2A:PP2A interactions in SHH or even other subtypes of medulloblastoma.

179. Time to Return to School Following 10 Common Orthopaedic Surgeries Among Children and Adolescents

Willimon, S. Clifton; Herzog, Mackenzie M.; Busch, Michael T.

Purpose: Many orthopeadic studies focus on a patient's ability to return to sport, and the important issue of return to school is often overlooked. The purpose of this study was to prospectively measure the time missed from school after 10 common orthopedic surgeries. Methods: School-aged patients, 5 to 19 years old, who underwent treatment for fixation of type III supracondylar humerus fracture, midshaft femur fracture fixation, isolated anterior cruciate ligament (ACL) reconstruction, isolated partial menisectomy, adolescent idiopathic scoliosis (AIS) fusion, closed reduction of both-bone forearm fracture, arthroscopic Bankart repair, hip arthroscopy with femoroacetabular impingement (FAI) correction, limb length discrepancy correction, surgical fixation of slipped capital femoral epiphysis (SCFE), or femur fracture fixation during the 2014/2015 and 2015/2016 school years were identified for this IRB-approved study. Patients in homeschooling and patients with neuromuscular disorder were excluded. All patients were contacted weekly following surgery to collect information about number of school days in session and number of school days missed. Demographic and injury information were also recorded, including name, date of birth, sex, ethnicity, school attended, grade level, date and type of injury. Return to school was defined by a physical attendance of school. The day of surgery was included in days missed. Patients with recovery extending into summer break were excluded. Results: 215 patients met the inclusion criteria for this study. Mean age at time of surgery was 13.0 +/- 3.8 years. There were 113 males (53%). Mean time to return to school for all surgeries was 12.8±14.9 days. Patients with AIS fusion or surgical fixation of SCFE typically took longer to return to school, where as patients with both-bone forearm or supracondylar fracture fixation took less time to return to school. Conclusion: Information regarding average time missed from school for 10 common orthopaedic surgeries can be used by clinicians to counsel patients and their families regarding expected recovery time. This information is also valuable for parents to plan for post-operative care of children undergoing orthopaedic surgery.

180. Applications of Hairpin DNA Functionalized Gold Nanoparticles For Molecular Identification of Disease

Wong, Alexis; Jackson, Stephen; Scherr, Thomas; Wright, David

Physicians routinely rely on microscopy, histopathology, or measurement of protein biomarkers for disease diagnosis, prognosis, and prediction. However, these techniques cannot report on the molecular status of a disease in real time. For example, examination of tissues by microscope does not reveal the

highly heterogeneous gene expression exhibited by cells within malignant tumors. Analysis of the molecular signature of these cells will support the prediction of disease progression and prognosis. Additionally, infectious diseases like Zika virus can be difficult to identify due to presence of coinfections, cross-reactivity in serological diagnostic tests, and mild symptoms similar to that of other infections. Therefore, convenient evaluation of gene expression in tumors and the molecular discrimination of infectious diseases remain important unmet needs in translational research. We have developed fluorescent hairpin DNA-functionalized gold nanoparticles (hAuNPs) as molecular probes for the detection of RNA. These probes are modular with respect to target molecule, are easily internalized by cells, and can be multiplexed. In this work, we demonstrate the utility of hAuNPs as intracellular mRNA probes by imaging of three matrix metalloproteinase mRNAs that have variable expression in three breast cancer cell lines. Furthermore, the process of cellular uptake of hAuNPs is explored to determine if nanoparticle size, shape, surface charge, or surface functionality affects the transport of these probes into cancer cell lines. We will demonstrate that the size of a DNA functionalized gold nanoparticle construct is the characteristic that most strongly determines the extent of internalization of these nanoparticles. These results suggest that DNA-AuNPs can be easily tailored through modulation of size to design functional AuNPs with optimal cellular uptake properties and enhanced performance in nanomedicine applications. Finally, we propose a silica-based magnetic bead extraction method for the purification and concentration of Zika virus RNA from the urine of pregnant women followed by detection with Zika virus-specific hAuNPs. This assay will be multiplexed to detect Zika, Chikungunya, and Dengue virus, which are difficult to distinguish by serological assay or symptoms alone. Molecular detection of these three viruses from urine would allow for rapid screening to identify pregnant women infected with Zika, whose fetuses are at risk of developing microcephaly.

181. T-cell Depletion Improves Diastolic Dysfunction in Mice with Uremic Cardiomyopathy

Winterberg, Pamela; Jiang, Rong; Wagner, Mary B.; Ford, Mandy

Background: Uremic cardiomyopathy is a significant cause of cardiovascular morbidity and mortality among patients with chronic kidney disease (CKD), but the underlying mechanisms are incompletely understood. Left ventricular hypertrophy (LVH) and diastolic dysfunction are recognized features of uremic cardiomyopathy in children with CKD. Patients with CKD accumulate pro-inflammatory T cells and we found that mice with uremic cardiomyopathy also have profound alterations in their T cell repertoire. T cells have recently been implicated in mediating the adverse cardiac remodeling during pressure overload heart failure in mice. In light of these recent findings, we aimed to determine whether T cells are necessary for the development of experimental uremic cardiomyopathy. Methods: Male 129X1/SvJ mice were randomized to undergo two-stage partial nephrectomy versus sham operations. CKD mice were further randomized to receive intraperitoneal injections of anti-CD3 antibody to deplete T cells or isotype control antibody beginning 5-6 days after induction of CKD and continuing every 3-4 days. Echocardiography was performed at 6 weeks of CKD to assess changes in LV geometry (relative wall thickness and LV mass) and diastolic function (trans-mitral flow index [E/A ratio], isovolumic relaxation time [IVRT], and myocardial performance index [MPI]). Flow cytometry (CD3, CD4, CD8, CD19) was performed to verify depletion of T cells in spleen, blood, and mediastinal lymph nodes. Blood pressures were measured using the tail-cuff method. Kidney function was assessed via measurement of plasma urea and cystatin C concentrations. Results: Treatment with anti-CD3 antibody successfully reduced CD4+ and CD8+ T cell populations in blood, spleen, and mediastinal lymph nodes of mice with CKD.

Measures of diastolic function including isovolumic relaxation time (IVRT: isotype 22.3 ± 2.28 vs anti-CD3 16.9 ± 2.25 ms; p<0.01), and myocardial performance index (0.63 ± 0.05 vs 0.48 ± 0.08; p<0.001) were normalized and trans-mitral flow index improved (E/A ratio: 0.9 ± 0.14 vs 1.2 ± 0.23; p<0.01) in CKD mice receiving depleting antibody treatment. However, measures of LV geometry, including LV mass and relative wall thickness were not significantly different between CKD mice with or without T cell depletion. Similarly, systolic blood pressure and renal function were similar between CKD mice. Conclusions: Depletion of T cells improved diastolic dysfunction in mice with CKD independent of blood pressure and kidney dysfunction. We are pursuing further work into the mechanisms by which T cells mediate diastolic dysfunction in uremic cardiomyopathy.

182. Induced Pluripotent Stem cells as a Potential Cell Source for Tissue Engineered Heart Valves
Yonezawa, Aline; Singh, Monalisa; Safranski, David; Dupont, Kenneth; Xu, Chunhui; Davis, Michael

Despite recent advances in tissue engineered heart valves (TEHV), one of the major challenges is finding a suitable cell source for seeding TEHV scaffolds. Native heart valves are durable because valve interstitial cells (VICs) maintain tissue homeostasis by synthesizing and remodeling the extracellular matrix. In this study, we demonstrate that induced pluripotent stem cells (iPSCs) can be derived into induced mesenchymal stem cells (iMSCs) using our feeder-free protocol and then further differentiated into VICs using a 3D cell culture environment. The differentiation efficiency was quantified using flow cytometry, immunohistochemistry staining, RT-PCR, and trilineage differentiation. In addition, iMSCs were encapsulated in polyethylene (glycol) diacrylate (PEGDA) hydrogels of varying stiffness, grafted with adhesion peptide (RGDS), to promote cell proliferation, remodeling, and further differentiation into VIC-like cells. VICs phenotype was characterized by the expression of α-SMA, vimentin, F-actin, and the ECM production after 7, 14, and 21 days. The results demonstrated that using our feeder-free differentiation protocol, iMSCs were differentiated from iPSCs. Our iMSCs had a 99.9% and 99.4% positive expression for MSC markers CD90 and CD44, respectively. As expected, there was 0.019% expression of CD45, which is a hematopoietic marker. In addition, iMSCs differentiated into adipogenic, chondrogenic, and osteogenic. When MSC derived cells were encapsulated in PEGDA hydrogels that mimic the leaflet modulus, we observed expression of α-SMA and F-actin after 7 days. Thus, the results from this study suggest that iPSCs can be a suitable cell source for TEHV by using a feeder-free differentiation approach and 3D culture.

183. Patterns of Visual Engagement Differ as a Function of Cognitive Profile in School-Aged Children with ASD
Yurkovic, Julia; Stallworthy, Isabella; Coben, Ella; Jones, Warren; Klin, Ami; Shultz, Sarah

Background: Heterogeneity is an obstacle to advancements in identifying and treating causes of autism spectrum disorder (ASD). Measures capturing the core underlying features of ASD, such as reduced interest in social stimuli, may provide a means for parsing phenotypic heterogeneity in ASD. For example, previous research has revealed that the social adaptive value of where children looked when viewing social scenes differed significantly based on IQ profile (Rice et al., 2012). The present study uses a novel approach for quantifying where a child is looking and their level of engagement with scene content. Objective: Examine whether visual engagement with scene content differs between subgroups of

ASD characterized by different cognitive profiles. Methods: School-age children with ASD watched socially relevant videos while eye-tracking data were collected. Participants were divided into four subgroups as in Rice et al. (2012): Participants with a verbal IQ (VIQ) advantage, a non-verbal IQ (NVIQ) advantage, an even IQ profile and higher full-scale IQ (FSIQ), and an even IQ profile and lower FSIQ. Viewer engagement was quantified by patterns of eye-blink inhibition, a method that capitalizes on the finding that people unconsciously adjust eye-blink timing to minimize the likelihood of missing critical information (Shultz et al. 2011). Probabilistically, people are least likely to blink when highly engaged with what they are viewing. Permutation testing identified Periods of statistically significant blink inhibition (indicating moments of high engagement) for each subgroup. Visual fixation time on eyes, mouth, body, and object regions were calculated for each child. Results: Comparisons of visual fixation revealed similar patterns of visual fixation between subgroups. In contrast, eye-blinking patterns revealed striking differences in when subgroups were engaged. Only 1.24% of highly engaging movie frames were perceived as engaging to all 4 subgroups whereas 75.76% of highly engaging frames were perceived as engaging by only one subgroup. These data suggest that subgroups likely experience these movies in very different ways. Ongoing analyses are aimed at further investigating the type of content that is perceived as engaging to each ASD subgroup. Conclusions: This study identified patterns of visual engagement in one of the largest eye-tracking samples of school-age children with ASD. Findings demonstrate that patterns of engagement with social content are influenced by the cognitive profile of children with ASD. These measures provide a promising means for parsing heterogeneity in ASD and represent an important step towards developing interventions tailored to an individual's learning style.

184. Moving Toward a Better Fontan Care Plan: the Utility of Interval Cardiac MRI
Zaki, Neil; Parks, W. James; Slesnick, Tim; McConnell, Michael; Oster, Matthew E.

Background: Gated cardiac MRI offers the most detailed and accurate noninvasive method of assessing cardiac anatomy, particularly in patients with complex congenital heart disease. The proposed benefits of using cMRI as a routine screening tool in the Fontan population include early recognition of asymptomatic, post-operative anatomic and physiologic changes. In 2011 we therefore instituted at our center a recommended practice of cMRI screening in patients with Fontan physiology at 3 and 8 years post-Fontan operation. The purpose of this study was to determine the impact of this standardized practice of cMRI screening on the clinical management of a Fontan population. Methods: We retrospectively reviewed charts from our institutional Fontan database to determine which patients were eligible for cMRI under the current guidelines and who underwent imaging from November 14, 2002 to June 25, 2015. We reviewed the frequency of obtaining cMRI and number of changes in management based on the results. Statistical significance was determined using a two-tailed mid-p exact test. Results: There were 34 out of 120 eligible patients who underwent cMRI at 3 years after Fontan. Six patients had changes in management due to 3 year cMRI findings. The most frequent changes included more frequent cMRI monitoring and cardiac catheterization for angioplasty or stenting. At 8 years post-Fontan, there were 43 out of 150 eligible patients who underwent cMRI. One patient had a change in management due to 8 year cMRI, leading to angioplasty. No patients have yet had both 3 and 8 year cMRI. P-value for changes in management is 0.03. Conclusions: Cardiac MRI at 3 years after Fontan completion appears to play an important role in informing the management of these medically complex patients. There appears to be less value in imaging at 8 years. Future studies of the Fontan care plan

should focus on increasing compliance with the recommended imaging studies, particularly at 3 years after the Fontan.

185. BAI1 Silencing Promotes Medulloblastoma Formation Through a P53 Dependent Mechanism
Zhu, Dan; Zhang, Zhaobin; Yang, Liquan; Osuka, Satoru; Devi, Narra; You, Shuo; Olson, Jeffrey; Van Meir, Erwin

Brain-specific Angiogenesis Inhibitor 1 (BAI1) is a seven transmembrane G protein-coupled receptor (GPCR) with potent anti-angiogenic and anti-tumorigenic properties. Here we investigated its physiological function in cerebellar transformation towards medulloblastoma (MB), the most malignant pediatric tumor. Methods: BAI1 expression in MBs was determined by analyzing micro array datasets, RT-PCR and immunohistochemistry (IHC). DNA methylation at the BAI1 promoter was determined by bisulfite-sequencing. Bai1-/- mice were generated by gene targeting and homologous recombination. Results: Here we show that BAI1 expression is significantly reduced in MBs. There is no copy number variation of the BAI1 gene on 8q24 in MB samples, but evidence for gene silencing through aberrant DNA and histone methylation at the Bai1 promoter. Furthermore, knockdown of the methyl-CpG–binding domain protein MBD2 and/or the histone methytransferase EZH2 is able to reactivate BAI1 expression in MB cells, which suggests lack of BAI1 expression in MBs is mediated by epigenetic mechanisms. To explore the function of BAI1 in MB tumorigenesis, we generated a Bai1-/- mouse line. Knockout of Bai1 in mice augments proliferation of granule neuron precursors (GNPs), and leads to a dramatic acceleration of tumor growth in the Ptch1+/- transgenic MB mouse model, which is associated with destabilization of Trp53. Restoration of BAI1 expression in MB cells stabilizes TP53 and inhibits cell proliferation, effects that are blocked by TP53 knockdown, suggesting that BAI1's tumor suppressor activity in MB is TP53-dependent. Mechanistically, we reveal that BAI1 binds MDM2 and prevents its E3 ligase activity towards TP53. Conclusions: Our results demonstrate that BAI1 is a novel bona fide physiological tumor suppressor in MB. Moreover, for the first time, our data reveal a direct crosstalk between adhesion GPCR and TP53 signaling, and provide the first causal relationship between adhesion GPCRs and cancer.

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