2021 Pediatric Research and Career Development Symposium

Tuesday, October 5, 2021
9:00 am – 5:00 pm

Abstract Book

Oral & Poster

Sponsored by the NICHD supported
Atlanta Pediatric Scholars Program, K12HD072245
## BASIC SCIENCE ABSTRACTS

### Oral Presentations

<table>
<thead>
<tr>
<th>Presenting Author</th>
<th>Abstract Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christina Caruso</td>
<td>B-1. Stiff Erythrocyte Subpopulations Biomechanically Induce Endothelial Inflammation in Sickle Cell Disease</td>
</tr>
<tr>
<td>Waitman Aumann</td>
<td>B-2. Impaired CALM-AF10 Leukemia Cell Proliferation by a SIX1/EYA2 Inhibitor</td>
</tr>
<tr>
<td>Maria Barbian</td>
<td>B-3. Antenatal Butyrate Supplementation Reduces Postnatal Gastrointestinal Injury in a Murine Model of Colitis</td>
</tr>
<tr>
<td>Seema Patel</td>
<td>B-4. Differences in Non-Major Histocompatibility Complex and F8 Genes Influence the B Cell Response to Factor VIII</td>
</tr>
</tbody>
</table>

### Poster Presentations

<table>
<thead>
<tr>
<th>Presenting Author</th>
<th>Abstract Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holly Bauser-Heaton</td>
<td>B-5. A 3D Bioprinted In Vitro Model of Pulmonary Artery Atresia to Evaluate Endothelial Cell Response to Microenvironment</td>
</tr>
<tr>
<td>Alexandre Cammarata-Mouchtouris</td>
<td>B-6. Epigenetic Regulation of Lung-recruited Pathological Neutrophils in Cystic Fibrosis</td>
</tr>
<tr>
<td>Amir Dashti</td>
<td>B-7. Pharmacokinetics of an Anti-Env Monoclonal Antibody Cocktail in SIV-infected ART-suppressed Rhesus Macaques Treated with the SMAC mimetic AZD5582</td>
</tr>
<tr>
<td>Deepak Kumar</td>
<td>B-8. T Follicular Helper Cell Expansion and Chronic T Cell Activation are Characteristic Immune Anomalies in Evans Syndrome</td>
</tr>
<tr>
<td>Adeola Michael</td>
<td>B-9. Chronic Inflammation Promotes Hepatic Liver Injury in a Humanized Mouse Model of Sickle Cell Disease</td>
</tr>
<tr>
<td>Loretta Reyes</td>
<td>B-10. Metabolic Profiling reveals Differential Platelet Mitochondrial Bioenergetics in Children with Chronic Kidney Disease</td>
</tr>
<tr>
<td>Shubin Shahab</td>
<td>B-11. LIN28B and LIN28B-networks Regulate Growth and Metastasis in Group 3 Medulloblastoma</td>
</tr>
<tr>
<td>Jenny Shim</td>
<td>B-12. YAP Suppresses HRK To Promote Therapy Resistance Under Tumor Environmental Stress in Neuroblastoma</td>
</tr>
<tr>
<td>Aisha Walker</td>
<td>B-13. Enhanced Expression of MicroRNA 494 (Mir-494) Induces Fetal Hemoglobin but Diminishes Hemoglobin Production During Erythropoiesis</td>
</tr>
</tbody>
</table>
### CLINICAL & OUTCOMES RESEARCH ABSTRACTS

#### Oral Presentations

<table>
<thead>
<tr>
<th>Presenting Author</th>
<th>Abstract Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patricia Zerra</td>
<td><strong>C-1. Complement in Pediatric COVID-19 Infection and Multisystem Inflammatory Syndrome in Children (MIS-C)</strong></td>
</tr>
<tr>
<td>Justin Yoo</td>
<td><strong>C-2. Complement Activation during Vaso-Occlusive PainCrisis in Pediatric Sickle Cell Disease</strong></td>
</tr>
<tr>
<td>Swati Bhasin</td>
<td><strong>C-3. Single Cell Transcriptomics Analysis Of Paired Pediatric T-ALL Samples Collected At Diagnosis And Following End Of Induction Therapy Reveals an MRD-Associated Stem Cell Signature</strong></td>
</tr>
<tr>
<td>Rachel Krieger</td>
<td><strong>C-4. Risk of Nitrous Oxide Gas Use in Children With Sickle Cell Disease and B12 Deficiency</strong></td>
</tr>
</tbody>
</table>

#### Poster Presentations

<table>
<thead>
<tr>
<th>Presenting Author</th>
<th>Abstract Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holly Edington</td>
<td><strong>C-5. Predictors and Outcomes of Immunoglobulin Supplementation in Children with B-Cell Acute Lymphoblastic Leukemia</strong></td>
</tr>
<tr>
<td>Kristina Gerencser &amp; Rachel Yosick</td>
<td><strong>C-6. Establishing Conditioned Reinforcers for Minimally Verbal Children with Autism Spectrum Disorder</strong></td>
</tr>
<tr>
<td>Madeleine Goldstein</td>
<td><strong>C-7. Examining Intersectional and HIV-related Stigma Among Youth Living with HIV in Atlanta, Georgia</strong></td>
</tr>
<tr>
<td>Robert Grell</td>
<td><strong>C-8. Feasibility of the Virtual Reality (VR) based Pediatric Display Enhanced Testing for Cognitive Impairment (PeDETECT) and mild Traumatic Brain Injury (TBI) tool to assess concussion in the pediatric emergency department (ED)</strong></td>
</tr>
<tr>
<td>Xu Ji</td>
<td><strong>C-9. Mental Health Needs in Parents of Children with Cancer: A Claims-based Analysis</strong></td>
</tr>
<tr>
<td>Kristy Rostad</td>
<td><strong>C-10. Clinical and Serological Responses to COVID-19 Vaccination in Children with History of MIS-C</strong></td>
</tr>
<tr>
<td>Ryan Summers</td>
<td><strong>C-11. Comprehensive Genomic Profiling of High-Risk Pediatric Cancer Patients has a Measurable Impact on Clinical Care</strong></td>
</tr>
</tbody>
</table>
B-1. Stiff Erythrocyte Subpopulations Biomechanically Induce Endothelial Inflammation in Sickle Cell Disease

Authors: Caruso, Christina; Zhang, Xiao; Sakurai, Yumiko; Li, Wei; Fay, Meredith; Carden, Marcus; Myers, David; Mannino, Robert; Joiner, Clinton; Graham, Michael; and Lam, Wilbur

Presenting Author: Christina Caruso, MD
Type: Basic - Oral

BACKGROUND: Originally described as a monogenic hemoglobin disorder resulting in increased red blood cell (RBC) stiffness leading to vaso-occlusion, sickle cell disease (SCD) is now known to be a vasculopathic disease with some semblance to cardiovascular disease in which the endothelium is dysfunctional and inflamed. Recent work has shown that leukocyte-endothelial interactions, inflammatory cytokines, and intravascular RBC destruction all contribute to SCD vasculopathy, but there remains much to learn, particularly regarding RBC-endothelial interactions. We hypothesize that aberrant forces applied by flowing sickle RBCs lead to increased abrasion or collision-like RBC-endothelial interactions that directly contribute to endothelial dysfunction in SCD in a biophysical manner distinct from microvascular occlusion.

METHODS: Using our microfluidic microvasculature models with human umbilical vein endothelial cells cultured throughout each microchannel, RBCs from SCD patients were “spiked” into normal RBC suspensions to comprise 5 and 10% of the overall population (a representation of irreversibly sickled cells in vivo), suspended in media to 25% hematocrit mimicking conditions seen in SCD patients, and perfused into the microfluidics. Samples of 100% normal RBCs or SCD RBCs were run in parallel. To isolate the stiffness effects of sickle RBCs without confounding hemolytic and adhesive effects, parallel experiments were conducted using nystatin-treated RBCs to create artificially stiffened RBC subpopulations, defined by elevated mean corpuscular hemoglobin concentrations (MCHCs), at the same proportion (0, 5, 10 and 100%). The endothelialized models were then immunostained with antibodies against VCAM-1 and E-selectin, known markers of endothelial cell inflammation, and mean fluorescence intensity was measured.

RESULTS: Endothelium exposed to 5, 10, and 100% SCD RBCs exhibited increased VCAM-1 and E-selectin expression over normal RBCs, and the degree of expression increased with higher percentages of SCD RBCs. While endothelial cells exposed to nystatin-stiffened RBCs also showed increased VCAM-1 and E-selectin expression, those exposed to a lower percentage of stiff cells (5 and 10%) exhibited higher expression than the homogenously stiff (100%) condition.

CONCLUSIONS: Purely non-adhesive, physical interactions between endothelial cells and SCD RBCs are sufficient to cause endothelial inflammation. Furthermore, heterogeneous RBC populations, comprised of a small minority of stiff cells, cause more inflammation than uniformly stiff RBCs.
B-2. Impaired CALM-AF10 Leukemia Cell Proliferation by a SIX1/EYA2 Inhibitor

Authors: Aumann, Waitman; Lavau, Catherine; Chen, Dongdong; Conway, Amanda; Ford, Heide; and Wechsler, Daniel

Presenting Author: Waitman Aumann, MD, MS

Type: Basic - Oral

Background: CALM-AF10, found in 5-10% of T-cell acute lymphoblastic leukemias (T-ALL), is characterized by CRM1 dependent overexpression of proleukemic HOXA genes. To discover other novel CALM-AF10-regulated genes, we used NGS to identify 11 genes that are both decreased in response to CRM1 inhibition and increased in response to CALM-AF10. Of these genes, SIX1 is a homeobox gene associated with embryogenesis and is quiescent post-embryologically. Additionally, SIX1 and its cofactor EYA2 are overexpressed in numerous solid tumors, and an inhibitor of the SIX1/EYA2 complex (Compound 8430) has recently been described. While there is evidence of a role for SIX1 in solid tumors, its role in leukemias has not been explored.

Objective: Evaluate the effect of a SIX1/EYA2 complex inhibitor on leukemia cell proliferation.

Design/Methods: SIX1 gene and protein expression were assessed in CALM-AF10, Jurkat (T-ALL) and NOMO1 (AML) leukemia cell lines. The effect of compound 8430, alone and in combination with the CRM1 Nuclear Export Inhibitor KPT-330, on cell proliferation was evaluated using Cell-Titer-Glo Assays and liquid culture proliferation assays.

Results: SIX1 gene and protein expression are increased in CALM-AF10 leukemia cell lines and Jurkat cells, but not NOMO1 cells. Compound 8430 decreased cell proliferation in CALM-AF10 leukemias and Jurkat leukemia cell lines, however not the NOMO1 cells. Correspondingly, liquid cultures showed 8430 alone slowed the proliferation of CALM-AF10 leukemia and Jurkat cells, but not NOMO1 cells. KPT-330 and 8430 are synergistic in CALM-AF10 leukemia cells with a KPT-330 dose of 60 nM and multiple dose levels of 8430, while in the Jurkat leukemia cells a dose of 30 nM of KPT-330 was synergistic at multiple dose levels of 8430.

Conclusions: Through an initial unbiased screen, we discovered Six1 may play a role in CALM-AF10 leukemogenesis. This role is further supported by the ability of a SIX1/EYA2 inhibitor to slow the proliferation of CALM-AF10 leukemia cells. Importantly, based on our observation that 8430 slows proliferation of Jurkat cells, SIX1 inhibition may be relevant in other leukemias. Finally, our demonstration that 8430 synergizes with KPT-330, a Nuclear Export Inhibitor, suggests the possibility of a novel therapeutic approach for CALM-AF10 and other leukemias.
**B-3. Antenatal Butyrate Supplementation Reduces Postnatal Gastrointestinal Injury in a Murine Model of Colitis**

**Authors:** Barbian, Maria; Owens, Joshua; Naudin, Crystal; Denning, Patricia; Patel, Ravi; and Jones, Rheinallt.

<table>
<thead>
<tr>
<th>Presenting Author:</th>
<th>Maria Barbian, MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type:</td>
<td>Basic - Oral</td>
</tr>
</tbody>
</table>

**BACKGROUND/PURPOSE:** The maternal diet during pregnancy can impact progeny health and disease, in part by influencing the offspring’s gut microbiome and immune development. Gut microbial metabolism generates butyrate, a short-chain fatty acid which has beneficial effects on intestinal health. Butyrate has been shown to enhance intestinal barrier function, regulate intestinal mucosal immunity and reduce intestinal inflammation. We sought to determine whether optimizing the antenatal diet, with butyrate supplementation, reduces the severity of intestinal injury in offspring with dextran sulfate sodium induced colitis. We hypothesize that antenatal butyrate supplementation will reduce the severity of intestinal injury in offspring with colitis.

**METHODS:** Wild type C57Bl/6 mice received butyrate supplementation during pregnancy. A series of experiments were then performed on their offspring at various stages of development. RNA sequencing was performed on colonic tissue of 3-week-old offspring. At 6 to 8-weeks of age, microbiome analysis was performed, and offspring were then subjected to a model of dextran sulfate sodium induced acute or chronic colitis.

**RESULTS:** Antenatal butyrate supplementation decreased the expression of pro-inflammatory genes in colonic tissue from 3-week-old offspring. Offspring exposed to antenatal butyrate had significantly lower colonic injury compared to controls in models of acute and chronic colitis. Antenatal butyrate supplementation increased the adult offspring’s gut microbiome alpha and beta diversity and caused expansion of specific gut microbes including Bifidobacteriaceae.

**CONCLUSIONS:** Our study highlights the importance of the antenatal diet on fetal gut development. Antenatal butyrate supplementation resulted in down regulation of colonic genes associated with inflammatory signaling, was associated with protection against acute and chronic colitis and an expanded microbiome diversity in 6 to 8-week-old offspring. These results suggest that antenatal butyrate has an impact on fetal gut development and this impact leads to lasting protective effects on the offspring’s postnatal gut health. Thus, antenatal butyrate supplementation may represent a therapeutic intervention for pregnant women to protect against future gut injury in their children.
B-4. Differences in Non-Major Histocompatibility Complex and F8 Genes Influence the B Cell Response to Factor VIII

Authors: Patel, Seema; Lundgren, Taran; Baldwin, Wallace Hunter; Cox, Courtney; Parker, Ernest; Stowell, Sean and Meeks, Shannon

Presenting Author: Seema Patel, PhD
Type: Basic - Oral

Introduction: Inhibitors (neutralizing antibodies) to factor VIII (fVIII) are a significant barrier for the treatment of hemophilia A patients. However, not all patients develop inhibitors, and of those that do, some form persistent titers and others a transient response. Though Human Leukocyte Antigen (HLA) variants and F8 gene mutations have been proposed to influence the immune response to fVIII, monozygotic twins and siblings with the same F8 mutation can form different outcomes, and HLA variants are weakly associated with risk of producing inhibitors. Thus, we tested the impact of non-Major Histocompatibility Complex (MHC) genes on the immunogenicity of fVIII.

Methods: Hemophilia A mice with the same F8 mutation and MHC haplotype (H-2b) but distinct backgrounds [129S4/SvJae + B6 (S129/B6) or B6] were administered four weekly infusions of 1 microgram fVIII, followed by a 2 microgram boost. One week before and after boost, plasma was collected for examination of anti-fVIII antibodies by an enzyme linked immunosorbent assay. Splenocytes were harvested one-week post boost to evaluate the B cell and CD4 T cell response using our novel tetramers.

Results: S129/B6 hemophilia A mice generated a statistically enhanced antibody and inhibitor response to fVIII compared to B6 hemophilia A mice. The observed differential response in S129/B6 and B6 hemophilia A mice was found to not be due to differences in the precursor frequency of fVIII specific B cells. Rather, S129/B6 hemophilia A mice demonstrated a more robust germinal center and class-switched B cell response to fVIII compared to B6 hemophilia A mice. Interestingly, this differential B cell response did not appear to be due to differences in the CD4 T cell response to fVIII, as S129/B6 and B6 hemophilia A mice produced a similar CD4 T cell response to the C2 domain of fVIII.

Conclusion: The discordant antibody response in S129/B6 and B6 hemophilia A mice is due to a differential B cell response to fVIII. As the CD4 T cell response appears to be similar between the mice, these results indicate that there are undefined non-MHC genes that may directly impact the pathway by which an antibody response is formed to fVIII.
B-5. A 3D Bioprinted In Vitro Model of Pulmonary Artery Atresia to Evaluate Endothelial Cell Response to Microenvironment

Authors: Tomov, Martin; Perez, Lilanni; Ning, Liqun; Chen, Huang; Jing, Bowen; Mingee, Andrew; Ibrahim, Sahar; Theus, Andrea; Kabboul, Gabriella; Do, Katherine; Bhamidipati, Sai Raviteja; Fischbach, Jordan; McCoy, Kevin; Zambrano, Byron; Zhang, Jianyi; Avazmohammadi, Reza; Mantalaris, Athanasios; Lindsey, Brooks; Frakes, David; Dasi, Lakshmi Prasad; Serpooshan, Vahid; and Bauser-Heaton, Holly

Presenting Author: Holly Bauser-Heaton, MD, PhD

Type: Basic - Poster

Background: Cardiovascular diseases are a major cause of morbidity and mortality and can have lifelong complications. In the case of vascular atresia due to congenital malformations or coronary total occlusions, treatment may consist of transcatheter recanalization or surgical vascular anastomosis. Currently, the cellular response to vascular anastomosis or recanalization is largely unknown and state-of-the-art techniques rely on restoration, rather than optimization of flow into the atretic arteries. An improved understanding of cellular responses post anastomosis may result in reduced restenosis of these complex vasculatures.

Methods: We used in vitro platforms to model anastomosis in complex architectures and for procedural planning to reduce vascular restenosis through optimization of geometry and flow patterns. A multimaterial bioprinting approach was used to create bifurcated vascular patterns within 3D hydrogel constructs to simulate a reestablished intervascular connection made via surgical or transcatheter techniques. The bifurcated models were seeded with human endothelial cells and perfused at physiologic flow rates to form an endothelium onto the luminal space. Flow hemodynamics in the fabricated constructs were analyzed using both particle image velocimetry and computational fluid dynamics modeling. Cell viability, proliferation, and metabolite bioprofiles of printed endothelialized vessels were also examined to identify the effect of geometry and flow on vascular cell behavior.

Results: We demonstrate that a previously validated 3D bioprinted model of pulmonary atresia can be used to study patterns of homeostatic flow rate and long-term cell behavior in a perfused and recanalized construct. Specifically, we show significant morphological changes in cell alignment under flow as compared to static controls and demonstrate consistent agreement between predictive models of flow and wall shear stress via computational flow dynamics with particle velocimetry data from our 3D printed constructs.

Conclusion: The combined in vitro and in silico methods establish a unique platform to study complex cardiovascular diseases that can serve as a live functional model and lead to direct clinical improvements in surgical planning and execution for vascular atresia and other diseases of disturbed flow. Agreement between fluid dynamic models (in silico) and velocimetry data (in vitro) of physical constructs opens up possibilities to better model disease onset and progression.
B-6. Epigenetic Regulation of Lung-recruited Pathological Neutrophils in Cystic Fibrosis

Authors: Cammarata-Mouchtouris, Alexandre; Moncada, Diego; Dobosh, Brian; Aldeco, Milagros; Scharer, Christopher; and Tirouvanziam, Rabindra

Presenting Author: Alexandre Cammarata-Mouchtouris, PhD
Type: Basic - Poster

Rationale: Massive inflammation is a hallmark of cystic fibrosis (CF) lung disease. Despite the presence of neutrophils known for their antibacterial function, patients with CF suffer from crippling bacterial infections. Our laboratory discovered that CF lung neutrophils undergo a reprogramming process that represses their bacterial killing activity, in a new fate we dubbed “GRIM”. We also established that GRIM neutrophils can be mass-produced in vitro by recruitment of blood neutrophils through a differentiated epithelial layer into sputum supernatant from CF patients (Forrest et al. 2018; Margaroli et al. 2021).

Approach: Using our in vitro model, we are performing transcriptional and epigenetic techniques to identify the dynamic reprogramming events leading to the GRIM neutrophils fate. Methods used include RNA-Seq to track genes expression, and ATAC-Seq to determine changes in the chromatin structure. In addition, we are repurposing small molecule epigenetic / transcriptional modulators to alter GRIM neutrophils phenotype and recover their bacterial killing function.

Results: We observed that epigenetic-related proteins are significantly modulated over time in GRIM neutrophils. In a pilot ATAC-Seq assay, we probed the epigenetic remodeling of chromatin and observed broad derepression in lung-recruited cells compared to blood controls. We also showed that targeted inhibition of a specific histone deacetylase (HDAC11) and a specific histone methyltransferase (EZH2) using repurposed cancer drugs reverses the GRIM fate in neutrophils, restoring their bacterial killing activity.

Conclusion and perspectives: Dynamic adaptation of lung-recruited neutrophils associated with human lung inflammation is regulated by epigenetic and transcriptional modulators, which is unexpected in these cells owing to the hypercondensed chromatin they display while in blood. Further experiments using CUT&RUN-Seq and DNA methylation profiling will bring further understanding into these processes and help identify novel epigenetic and transcriptional targets for treatment of CF and other intractable lung diseases via fate modulation of tissue neutrophils.

Funding: Cystic Fibrosis Foundation (TIROUV19G0, CAMMAR21F0), Emory I3 Team Award.
**B-7. Pharmacokinetics of an Anti-Env Monoclonal Antibody Cocktail in SIV-infected ART-suppressed Rhesus Macaques Treated with the SMAC mimetic AZD5582**

**Authors:** Dashti, Amir; Sukkestad, Sophia; Horner, Anna Marie; Neja, Margaret; Schoof, Nils; Vanderford, Thomas; Liang, Shan; Mavigner Maud; Lifson, Jeffrey D; Dunham, Richard M; Tuyishime, Marina; Ferrari, Guido; Mason Rosemarie D; Roederer, Mario; Silvestri Guido; Margolis David M; and Chahroudi, Ann

<table>
<thead>
<tr>
<th>Presenting Author</th>
<th>Amir Dashti, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Basic - Poster</td>
</tr>
</tbody>
</table>

**Background:** The use of neutralizing and binding antibodies holds promise as a clearance mechanism to reduce infected cells following effective latency reversal. Here, we evaluated a cocktail of four SIV-specific monoclonal antibodies (mAbs) in ART-suppressed rhesus macaques (RMs) treated with AZD5582 to reverse latency by activating the non-canonical NF-kB pathway.

**Methods:** 15 RMs were infected i.v. with SIVmac239 and ART was initiated 8 wks later. After 90 wks of ART, 9 RMs received a cocktail of 4 anti-SIV Env mAbs: ITS-09, ITS-102, ITS-103, ITS-113. mAbs were administered s.c. at 20 mg/kg each followed by AZD5582 at 0.1 mg/kg i.v. weekly for 5 wks; this cycle was repeated once. 3 RMs received the mAb cocktail only and 3 RMs served as controls. ART was continued in all RMs. ELISAs to measure mAb levels and anti-drug antibodies (ADA) were performed.

**Results:** Peak viremia of 107-108 copies/ml occurred two wks after SIVmac239 infection and ART was successful in suppressing viral loads. Infused antibodies were detectable in all 12 RMs 24h after infusion with Cmax levels of 289, 415, 325, and 814 ug/ml for ITS-09, -102, -103, and -113, respectively. Ctrough levels prior to the 2nd mAb infusion were 51.26 (ITS-09), 25.99 (ITS-102), 10.06 (ITS-103), and 102.36ug/ml (ITS-113). Cmax levels after the 2nd mAb infusion were similar to the first, except ITS-103 (180 ug/ml). In 7/9 RMs given the mAb cocktail in combination with AZD5582, we observed ADA against ITS-103 with serum concentration of this mAb dropping to undetectable for ≥1 weekly measurement in 4/7. ADA was not observed in the 3 RMs who received the mAb cocktail alone. On-ART viremia > 60 copies/ml was consistently detected in 7/9 AZD5582-treated RMs.

**Conclusions:** Development of ADA against some anti-Env mAbs was more common in RMs treated with AZD5582 suggesting that the events triggering virus reactivation may also stimulate the immune system. When ADA was not observed, mAb levels remained high for 6 weeks and in all cases could be boosted by the 2nd infusion. Combination antibody therapy, as used here, may prove beneficial for 'kick and kill' cure approaches to minimize unwanted adverse immune effects.
B-8. T Follicular Helper Cell Expansion and Chronic T Cell Activation are Characteristic Immune Anomalies in Evans Syndrome

Authors: Kumar, Deepak; Prince, Chengyu; Bennett, Carolyn M; Briones, Michael; Lucas, Laura; Russell, Athena; Patel, Kiran; Chonat, Satheesh; Graciaa, Sara; Edington, Holly; White, Michael H.; Kobrynski, Lisa; Abdalgani, Manar; Parikh, Suhag; Chandra, Sharat; Blesing, Jack; Marsh, Rebecca; Park, Sunita; Waller, Edmund K.; Prahalad, Sampath and Chandrakasan, Shanmuganathan

Presenting Author: Deepak Kumar, PhD
Type: Basic - Poster

Background: Pediatric Evans syndrome (pES) is increasingly identified as the presenting manifestation of primary immune deficiency and immune regulatory disorders. Genetic defects in genes associated with primary immune deficiency disorders are found in 30-40% of pES cases. Despite an improved understanding of the genetic basis of pES, the underlying immunobiology of pES is poorly defined, and characteristic immune diagnostic parameters are still lacking. This study aims to identify characteristic immune abnormalities of pES patients and further our understanding on the immune pathobiology of pES.

Methods: To delineate the immune biology of pES, we performed high dimensional flow cytometry, gene expression profiling, T cell repertoire evaluation and plasma cytokine profiling of 24 pES patients and compared the immune profile of pES with 22 chronic immune thrombocytopenia (cITP) and 24 healthy controls.

Results: We found that pES is characterized by marked expansion of circulating T-follicular helper cells (cTfh), chronic T cell activation, and decreased naïve CD4+ T cells for age. Despite normal or high IgG in the majority of pES at presentation, class-switched memory B cells (CSMB) were decreased. Within the cTfh subset, we noted features of post-activation exhaustion with upregulation of several canonical checkpoint inhibitors. TCR-β repertoire analysis of cTfh cells revealed increased oligoclonality in patients with pES compared with HC. Among patients with pES, those without a known gene defect had a similar characteristic immune abnormality as patients with defined genetic defects. Similarly, patients with pES with normal IgG had similar T cell abnormalities as patients with low IgG. Since genetic defects have been identified in only less than half of patients with pES, our findings of similar immune abnormalities across all pES patients help establish a common characteristic immunopathology in pES irrespective of the underlying genetic etiology.

Conclusions: These findings define the characteristic shared immune abnormality in patients in pES irrespective of underlying genetic drivers and functional status of B cells. Expanded cTfh, low naïve CD4 and CSMB, and increased T cell activation are unique features of pES and distinguish pES from cITP.
B-9. Chronic Inflammation Promotes Hepatic Liver Injury in a Humanized Mouse Model of Sickle Cell Disease

Authors: Michael, Adeola O. Adebayo; Flowers-Steele, Jalesia; Song, Hannah; Archer, David and Platt, Manu

Presenting Author: Adeola Michael, PhD
Type: Basic - Poster

Sickle cell disease (SCD) is one of the most common genetic diseases in the world. Liver or biliary complications including hepatomegaly, cirrhosis, chronic biliary disease, and fibrosis is evident in about 40% of hospitalized SCD patients. However the molecular mechanisms responsible for sickle cell hepatobiliary injury remains poorly understood.

We hypothesized that the chronic inflammatory state associated with SCD promotes hepatobiliary injury and can be modeled in SCD mice. Methods: Townes humanized sickle cell mice were kept and bred in climate-controlled rooms and are allowed access to food and water ad libitum. At 3-months of age, liver tissue and blood were collected at necropsy for histologic and biochemical analysis.

Results: Male and female SS mice had increased liver size compared to AA controls. Focal necrosis, portal fibrosis, micronodular cirrhosis, with intrahepatic sequestration and lymphocytic infiltration were observed in SS mice liver compared to controls. There was a statistically significant increase in liver weight to body weight ratio, and evaluation of liver histology by H&E revealed increased portal bridging and red blood cells in the sinusoids of SS mice compared to AA control mice. Serum ALT, AST, total and direct bilirubin levels were markedly increased in SS mice compared to AA controls. Collagen deposition was increased as measured by Sirius Red staining and increased α-SMA expression in SS mice compared to AA controls. We observed significantly increased hepatic mRNA gene expression of proinflammatory markers: IL6, TNF-a, and IL1a, increased leukocytes markers: CD45 (pan-leukocytes), CD3 (T-cells), CD11b (neutrophils), F4/80 (macrophages) and increased hepatic stellate cell markers: cytoglobin and GFAP in SS mice compared to AA controls. We observed significantly increased hepatic expression of phosphorylated IKBβ protein levels and increased active enzymes cathepsins in SS mice.

Conclusion: These observations indicate that the chronic inflammatory milieu of SCD promotes hepatic injury in the mice model of SCD. Decreased hepatic functionality and increased collagen deposition in the SCD mice model supports clinical data of hepatobiliary dysfunction in patients. Our findings suggest that the SCD mouse will be a useful model to study the pathogenesis of SCD-associated liver disease.
B-10. Metabolic Profiling reveals Differential Platelet Mitochondrial Bioenergetics in Children with Chronic Kidney Disease

Authors: Reyes, Loretta; Smith, Matthew; Khanna, Anjali; Wilkerson, Alexandria; Harris, Frank; Morris, Claudia; and Sutliff, Roy

Presenting Author: Loretta Reyes, MD
Type: Basic - Poster

Background: Chronic kidney disease (CKD) is a systemic disease associated with increasing complications as kidney function declines. Oxidative stress, characterized by overproduction of reactive oxygen species (ROS) and/or reduction in antioxidant defense capacity, has been implicated in the pathogenesis of CKD progression and its complications. ROS can originate from superoxide-generating enzymes and the mitochondrial respiratory chain and recent advances have led to a greater appreciation of the contribution of mitochondrial dysfunction to the pathogenesis of systemic diseases. Circulating platelets in peripheral blood have fully functional mitochondria that can serve as surrogate markers of systemic mitochondrial function; there is increased data on the assessment of platelet mitochondrial bioenergetics in systemic disease. We sought to identify differences the mitochondrial bioenergetic profile in children with mild to moderate CKD and determine corresponding metabolic alterations.

Methods: Children were classified according to their CKD stage (1, 2 and 3). Platelets were isolated by differential centrifugation of whole blood in the presence of PGI2, and isolated intact platelets seeded in a multi-well plate format. Mitochondrial bioenergetic function was examined using the Agilent Seahorse extracellular flux analysis platform with results normalized to the number of platelets seeded. Extracellular oxygen tension was measured in real time with subsequent serial additions of pharmacologic modulators (oligomycin, FCCP and rotenone/antimycin A) that enable calculation of parameters of mitochondrial respirometric function. Arginase activity and plasma markers of oxidative stress were measured by colorimetric assay and ELISA respectively. Targeted metabolomics of amino acid metabolism involved in oxidative stress and energy metabolism were conducted via LC-MS/MS.

Results: Across CKD groups, differences were seen in the mitochondrial bioenergetic profile. CKD 3 demonstrated a higher basal respiration, maximal respiration, spare reserve capacity and ATP production compared to CKD 1 and 2 (p=<0.001). CKD 3 also demonstrated lower proton leak compared to CKD 1 and 2 (p=<0.001). Trends toward statistical significance were noted in several amino acid profiles. There were no significant differences in arginase activity or measures of oxidative stress across CKD groups.

Conclusion: Heterogeneity was observed in the mitochondrial respirometric profiles in children with different stages of CKD. Potential metabolic drivers are currently being elucidated.
B-11. LIN28B and LIN28B-networks Regulate Growth and Metastasis in Group 3 Medulloblastoma

Authors: Shahab, Shubin; Rokita, Jo Lynne; Juraschka, Kyle; Kumar, Sachin; Taylor, Michael; Schneppe, Robert; MacDonald, Tobey; and Kenney, Anna.

Presenting Author: Shubin Shahab, MD, PhD
Type: Basic - Poster

Medulloblastoma (MB) is the most common pediatric malignant brain tumor and is currently divided into WNT, SHH, Group 3 and Group 4 subtypes. Even with multimodal chemotherapy, radiotherapy and surgery, many children with Group 3 MBs do not survive. We have previously demonstrated an oncogenic role for the RNA-binding protein (RBP) LIN28B in neuroblastoma. LIN28B is a key regulator of let-7 family miRNAs, which in turn inhibit LIN28A/B and other oncogenes. LIN28B has also been found to be upregulated in Wilms tumor, hepatoblastoma, germ cell tumors, leukemia among others. We hypothesize that LIN28B plays an important role in Group 3 MB and that a better understanding of LIN28B and LIN28B-driven networks will reveal novel therapeutic vulnerabilities. LIN28B levels are highest in Group 3 MB patients, and its overexpression is associated with significantly worse survival. Here we demonstrate that down-regulation of LIN28B using shRNA results in significant reduction in cell proliferation by CellTiter-Glo and increased apoptosis by Caspase-Glo (as well as by Annexin V assay; p<0.05). In contrast overexpression of LIN28B increases Group 3 cell proliferation and tumor sphere formation. Injection of LIN28B knockdown cells orthotopically into immunocompromised mice also leads to longer survival compared to control injected mice (p<0.005). We also use a small molecule inhibitor of LIN28B ‘compound 1632’ to demonstrate significant reduction in G3 MB cell proliferation. In addition, we find that PDZ-binding kinase (PBK) a downstream target of LIN28B is downregulated when LIN28B is depleted. PBK knock down also leads to decreased proliferation of Group 3 MB cells. Finally, RNA-seq profiling of patient derived cells following LIN28B depletion reveals LIN28B plays a role in regulation of epithelial-to-mesenchymal transition in G3 MB and is supported by immunoblots for EMT markers as well as transwell migration assay. This work will help define the role for LIN28B in Group 3 MB aggressiveness and establish LIN28B and LIN28B-driven networks as novel therapeutic targets in these patients.
**B-12. YAP Suppresses HRK To Promote Therapy Resistance Under Tumor Environmental Stress in Neuroblastoma**

**Authors:** Shim, Jenny; Lee, Jasmine Y; Jonus, Hunter C; Arnold, Amanda; Schnep, Robert W; Janssen, Kaitlyn M; Maximov Victor; and Goldsmith, Kelly C

<table>
<thead>
<tr>
<th>Presenting Author:</th>
<th>Jenny Shim, MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type:</td>
<td>Basic - Poster</td>
</tr>
</tbody>
</table>

**Background**

High risk neuroblastomas (NB) display more genomic alterations at relapse than at diagnosis, including increased transcriptional activity of the Yes-Associated Protein (YAP). YAP is a transcriptional co-activator that binds to TEAD family transcription factors, regulating genes associated with organ growth, cell renewal, and survival. While increased YAP gene signatures in NB portend a poor prognosis, its role in NB continues to be explored as a potential therapeutic target. We further delineate YAP’s role in NB and define a novel relationship between YAP and the pro-apoptotic BH3-only protein, Harakiri (HRK).

**Methods**

We evaluated YAP expression in patient-derived xenografts (PDXs) from the same patient’s diagnostic and post-therapy/relapsed NB tumor. Neuroblastoma cells (harboring RAS mutations) with YAP knockdown (shRNA) or YAP overexpression were used to assess proliferation in vitro, tumor growth in vivo, and chemotherapy or MEK inhibitor (trametinib) response. RNA sequencing of SK-N-AS shYAP and control cells was performed and analyzed focusing on differentially expressed genes. NLF shYAP and control cells were subjected to normal or serum-starved conditions and HRK expression and apoptosis were quantified.

**Results**

YAP expression and transcriptional activity are increased in relapse/post-therapy compared to diagnostic matched NB PDXs. YAP genetic inhibition does not affect cell proliferation or therapy response in vitro, yet strongly deters NB tumor growth and sensitizes xenografts to chemotherapy and trametinib in vivo. RNA sequencing showed that YAP significantly suppresses HRK, a BH3 pro-death protein known to activate mitochondrial apoptosis in response to tumor environment stress conditions, such as nutrient deprivation. HRK expression and intrinsic apoptosis significantly increase in response to serum starvation only when YAP is genetically inhibited. Importantly, HRK is decreased in the relapsed and post-therapy NB PDX compared to the paired diagnostic and vehicle-treated PDX respectively, validating this reciprocal YAP-HRK relationship in vivo.

**Conclusion**

YAP may drive the aggressive nature of recurrent neuroblastoma through modulation of in vivo stress conditions, promoting tumor growth and therapy resistance via inhibition of HRK. This supports YAP as a logical therapeutic target and HRK as novel tumor suppressor in neuroblastoma. Further efforts to understand the mechanism underlying YAP-mediated regulation of HRK may reveal therapeutic opportunities.
B-13. Enhanced Expression of MicroRNA 494 (Mir-494) Induces Fetal Hemoglobin but Diminishes Hemoglobin Production During Erythropoiesis

Authors: Walker, Aisha; Schrott, Valerie; Crosby, Danielle; Ofori-Acquah, Solomon; and Gladwin, Mark

| Presenting Author: | Aisha Walker, PhD, MPH |
| Type:             | Basic - Poster |

Increasing fetal hemoglobin (HbF) in patients with sickle cell disease (SCD) improves pathophysiology and clinical outcomes. MicroRNAs (miRNA) that target BCL11A and other repressors of gamma-globin to enhance HbF may offer new therapeutic approaches for SCD. We previously reported that miR-494 is up-regulated in erythroid cells of SCD patients treated with the HbF inducing drug, hydroxyurea. Here, we investigated whether modulation of miR-494 induces gamma-globin expression to increase HbF as a potential therapeutic target. We measured endogenous miR-494, gamma-globin and BCL11A expression by QPCR and western blot in the Townes knock-in mouse model of SCD during the developmental hemoglobin switch. In the spleen, a primary site of murine hematopoiesis, miR-494 decreases 2-fold by postnatal day 4 and remains low as the animal ages. This decline is concomitant with a significant 2-fold decline in gamma-globin mRNA, and 21-fold decline in gamma-globin protein. To determine whether miR-494 can modulate HbF, we created stable cell lines of Human Umbilical Cord Blood-Derived Erythroid Progenitor (HUDEP-2) cells using the shMIMIC lentiviral vector to overexpress miR-494 (HUD-494) or a non-targeting miRNA sequence (HUD-NT). We found that gamma-globin mRNA transcripts increased more than 2-fold in HUD-494 cells compared to control HUD-NT (P=0.002) by orthochromatic erythroblasts stage of differentiation. Because BCL11A is a predicted target of miR-494, we measured BCL11A protein by western blot and found a significant 33% reduction in BCL11A in HUD-494 compared to control (P=0.002). Unexpectedly, total hemoglobin measured by UV/Vis spectrophotometry was lower in HUD-494 cells compared to HUD-NT (464 pmol/cell vs. 700 pmol/cell; P=0.0035). We found no differences in erythroid differentiation as determined by glycophorin A erythroid marker. These results demonstrate that down-regulation of miR-494 is associated with developmental hemoglobin switching and enhanced expression of miR-494 increases HbF in erythroid progenitors. The corresponding decline in BCL11A protein suggests that miR-494 targets this gamma-globin repressor. The diminished hemoglobin with miR-494 overexpression suggests a broader role for miR-494 and limits the potential of this miRNA as a therapeutic target for SCD. Additional studies are needed to fully elucidate the role of miR-494 in erythropoiesis and hemoglobin homeostasis to determine its therapeutic potential for other conditions.
B-14. F5-Atlanta: A novel mutation in F5 associated with enhanced East Texas splicing and FV-short production

Authors: Zimowski, Karen; Petrillo, Teodolinda; Ho, Michelle D; Wechsler Julie; Shields Jordan; Denning, Gabriela Denning; Jhita, Navdeep; Rivera, Angel A; Escobar Miguel A; Kempton, Christine L; Camire, Rodney M; and Doering Christopher B.

Presenting Author: Karen Zimowski, MD
Type: Basic - Poster

Background: Elucidating the molecular pathogenesis underlying the East Texas Bleeding Disorder (ET) led to the discovery of alternatively spliced F5 transcripts harboring large deletions within exon 13. These alternatively spliced transcripts produce a shortened form of coagulation factor V (FV) in which a large portion of its B-domain is deleted. These FV isoforms bind tissue factor pathway inhibitor alpha (TFPIα) with high affinity, prolonging its circulatory half-life and enhancing its anticoagulant effects. While 2 missense pathogenic variants highlighted this alternative-splicing event, similar centrally-deleted FV proteins are found in healthy controls.

Methods & Results: We identified a novel heterozygous 832 base pair deletion within F5 exon 13, termed F5-Atlanta (F5-ATL), in a patient with severe bleeding. Assessment of patient plasma revealed markedly elevated levels of total and free TFPI and a truncated FV isoform, similar in size to the FV-short described in ET. Sequencing analyses of cDNA revealed the presence of a transcript alternatively-spliced using the ET splice sites, thereby removing the F5-ATL deletion. RNAseq using the F5-ATL patient’s leukocytes revealed that ET-spliced transcripts represent ~50% of the total F5 mRNA pool, and mapping of these ET-spliced transcripts demonstrated that they originate from the F5-ATL allele. This alternative-splicing pattern was recapitulated by in heterologous mammalian cells.

Conclusions: These findings support a mechanistic model consisting of cis-acting regulatory sequences encoded within F5 exon 13 that control alternative splicing at the ET splice sites. Alterations in this region of F5 may be distinctly involved in modulating the TFPI anticoagulant pathway. This work implies that any formerly ascribed benign polymorphisms within this region may in fact have functional significance. It also suggests the presence of a novel axis for controlling hemostatic balance.
C-1. Complement in Pediatric COVID-19 Infection and Multisystem Inflammatory Syndrome in Children (MIS-C)

Authors: Zerra, Patricia E.; Verkerke, Hans; McCoy, James; Jones, Jayre; Lu, Austin; Hussaini, Laila; Anderson, Evan J.; Rostad, Christina A.; Stowell, Sean R.; and Chonat, Satheesh

Presenting Author: Patricia Zerra, MD

Type: Clinical - Oral

SARS-CoV-2 related multisystem inflammatory syndrome in children (MIS-C) can lead to serious and long-term complications. The hyperinflammation and extreme cytokine response seen in MIS-C suggests excessive and dysregulated activation of the innate immune system. SARS-CoV-2 infection has been shown to drive local and systemic complement pathway (CP) hyper-activation, and recent case reports have successfully used complement inhibitors in the treatment of severe COVID-19 lung injury. Elucidating the complement-mediated immune mechanisms underlying hyperinflammatory syndromes could provide further insights and targeted treatment in MIS-C.

We hypothesize that unrestrained dysregulation of the CP caused by SARS-CoV2 infection is responsible for acute COVID-19 organ injury and contributes to MIS-C in pediatric patients.

To investigate the role of CP in SARS-CoV-2-related multi-organ injury, blood samples from pediatric patients with acute COVID-19, MIS-C and healthy controls were subjected to comprehensive analyses of all three CP. We noticed significant elevation in levels of anaphylatoxin C3a (p=0.0024) and C5b9 (p=0.0395) in patients with MIS-C as compared to acute COVID infection suggesting proximal and terminal CP activation. Other activation markers were less significantly different; C5a (p=0.0744), Bb (alternative CP p=0.210), while we noticed no difference between mannose binding lectin and classical CP. Next, we examined the role of factor H, the main regulatory protein of the CP. We found that patients with MIS-C had significantly higher levels of Factor H autoantibodies (FHAA, p<0.0001) when compared to patients with acute COVID-19 infection, suggesting that complement regulation may be impaired in these patients. These FHAA titers correlated with the severity of MIS-C.

In summary, we have for the first time shown evidence of both complement activation and complement dysregulation in pediatric patients with COVID-19 and MIS-C. Future studies are targeted to validate this role of FHAA using functional assays, and these studies may provide additional clues to reveal the pathogenesis of other hyperinflammatory syndromes such as Kawasaki disease. Simultaneously, we believe our preliminary data would be helpful to investigate the use of specific complement and immunomodulatory agents in pediatric patients with COVID-19 infection and MIS-C.
C-2. Complement Activation during Vaso-Occlusive Pain Crisis in Pediatric Sickle Cell Disease

Authors: Yoo, Justin; Graciaa, Sara; Jones, Jayre; Zuo, Zoey; Arthur, Connie; Leong, Traci; Joiner, Clinton; Stowell, Sean; and Chonat, Satheesh

<table>
<thead>
<tr>
<th>Presenting Author:</th>
<th>Justin Yoo, MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type:</td>
<td>Clinical - Oral</td>
</tr>
</tbody>
</table>

Background: Sickle cell disease (SCD) affects millions of individuals worldwide with substantial morbidity and mortality. Amongst acute complications in SCD, vaso-occlusive pain crisis (VOC) is the leading cause of hospitalization, with supportive care being the primary approach to management. Our group has demonstrated important contributions of complement pathway (CP) to the pathophysiology of SCD. This study prospectively analyzed the extent of CP activation among children with SCD presenting with VOC.

Methods: Patients with SCD requiring intravenous opioids for VOC were enrolled in an IRB-approved research study. Blood samples were collected within 48 hours of VOC presentation, and steady-state levels were obtained at a 4-week clinic follow-up.

Results: Sixty-four patients have been enrolled thus far, of which 43 (67%) had steady-state samples collected. Seventeen patients had paired complement work-up performed during acute and steady-state, and 4 of them had additional samples collected during subsequent VOC. Complement protein levels C3, C4, C5, properdin, factor B, and complement regulatory proteins factor H and I were unremarkable during VOC and steady-state. However, CP activation markers, specifically C3a, C5a, Bb, and C5b9 were significantly elevated during VOC compared to steady-state (p=0.0018,<0.0001, 0.0123 and 0.0915 respectively), suggesting activation of alternative and common CP during VOC. Remarkably, patients who re-presented with acute VOC exhibited similar increases in their anaphylatoxins C3a/C5a, substantiating the increases related to their VOC. Hemoglobin and LDH were similarly significant, suggestive of intravascular hemolysis. Three (7.1%) patients developed acute chest syndrome, two of whom experienced respiratory failure, and all exhibited significant CP activation. The area under the curve (AUC) of the ROC curve was analyzed to determine the ability of complement biomarkers to differentiate VOC from steady-state. Based on the AUC of these biomarkers, complement anaphylatoxins C3a and C5a exhibited the highest AUC of 0.76 and 0.87, respectively.

Discussion: These preliminary findings for the first time suggest that increased activation of the alternative and common CP is present in a large proportion of patients during VOC, and is associated with intravascular hemolysis. Specifically, C3a/C5a could not only predict disease activity during VOC, but provide pharmacological targets in patients during VOC.
C-3. Single Cell Transcriptomics Analysis Of Paired Pediatric T-ALL Samples Collected At Diagnosis And Following End Of Induction Therapy Reveals an MRD-Associated Stem Cell Signature

Authors: Bhasin, Swati S.; Thomas, Beena E.; Summers, Ryan J.; Sarkar, Debasree; Mumme, Hope; Mansour, Mohammed E.; Pilcher, William J.R.; Park, Sunita I.; Castellino, Sharon M.; DeRyckere, Deborah; Bhasin, Manoj; and Graham, Douglas K.

<table>
<thead>
<tr>
<th>Presenting Author:</th>
<th>Swati Bhasin, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type:</td>
<td>Clinical - Oral</td>
</tr>
</tbody>
</table>

Introduction

Despite recent improvement in outcomes for de novo disease, pediatric T-cell acute lymphoblastic leukemia (T-ALL) remains challenging to treat at relapse. Investigation into genomic markers of treatment response and therapy resistance offers an opportunity to further enhance outcomes for these patients. We previously identified a T-ALL blast-associated gene signature at diagnosis (Dx) and characterized the immune microenvironment in Dx T-ALL marrow samples using single cell transcriptome analysis (Bhasin et al. Blood 2020(ASH)). This approach allowed us to generate a granular expression map of both the T-ALL landscape and the Dx bone marrow (BM) immune microenvironment. Here we have studied samples collected from the same patients at Dx and End of Induction (EOI). The use of paired samples provides insight into treatment-induced changes in the microenvironment. Further, the inclusion of both minimal residual disease (MRD) positive and MRD negative samples allowed us to compare differences between these groups.

Results

Using our previously described blast-associated gene signature (Bhasin et al. ASH 2020) we were able to identify residual blast populations at EOI in MRD-positive samples. Comparative analysis of gene profiles at Dx and EOI showed significant changes in the microenvironment cell populations. Analysis of the EOI samples demonstrated the presence of patient specific blast cells in MRD positive samples. Analysis of communication networks between different cell types based on receptor and ligand expression levels between different cell types identified a CD34+ cluster of stem cells that had different interactions with other immune populations in the MRD positive and negative subsets. Differential expression analysis between the MRD positive and MRD negative cells in this CD34+ stem cell cluster identified higher expression of myeloid associated genes such as CEBPB, CEBPD, AZU1 in the MRD negative group relative to the MRD positive cells, which showed higher expression of B-cell related genes such as IGHM, VPREB1, CD79A/ B along with upregulation of P13K signaling in B-lymphocytes, B-cell receptor signaling and autophagy pathways. Analysis of upstream regulators demonstrated upregulation of MYC and TCF3 activity and inhibition of TGFB1, CSF3 and CEBPA in MRD positive compared to MRD negative samples.
C-4. Risk of Nitrous Oxide Gas Use in Children With Sickle Cell Disease and B12 Deficiency

Authors: Krieger, Rachel; Brown, Lou Ann; Dampier, Carlton; Harris, Frank; Manoranjithan, Shaminy; Mendis, Reshika; Cooper, Nicholas; Figueroa, Janet; and Morris, Claudia R

Presenting Author: Rachel Krieger, DO, MS
Type: Clinical - Oral

Background: The prevalence of B12-deficiency in children with sickle cell disease (SCD) is unknown, however B12-deficiency has been reported in 18% of SCD-adults versus 10% in patients without SCD (Kamineni 2006). A higher frequency of B12-deficiency in SCD may be due to higher rates of hemolysis, erythrocyte turnover and folate deficiency. Nitrous oxide gas is commonly used for dental procedures and is standard therapy for sickle-related vaso-occlusive-pain in France. Recently we reported acute resolution of sickle-related priapism with nitrous therapy (Greenwald 2019). Although nitrous is generally considered safe, patients with B12-deficiency can experience serious neurologic complications, as nitrous impacts cobalamin metabolism. This study evaluates B12 status in children with SCD.

Methods: Urine samples were prospectively collected as part of a randomized-controlled trial of parenteral arginine therapy in children with SCD requiring admission for treatment of moderate-to-severe pain. Urine methylmalonic acid (MMA) level corrected for creatinine (Cr) reflecting B12 status, was measured via mass spectrometry. B12 deficiency was defined as MMA/Cr of 2.2-5, while severe B12 deficiency was reflected by MMA/Cr>5; MMA/Cr of 1.8-2.2 were considered possibly deficient while an MMA/Cr<1.8 was defined as normal B12 status.

Results: Ninety-four children with SCD and pain were enrolled. Median age was 13 years (Q1, Q3: 10, 16), 51% female, 68% Hb-SS, and 71% were on hydroxyurea. Twenty-six percent (24/94) of patients demonstrated evidence of B12 deficiency, 25% of whom demonstrated a severe B12 deficiency (6/24). Another 7% (7/94) demonstrated possible deficiency. There were no statistically significant differences in demographics, SCD genotype, hemoglobin levels, MCV or hydroxyurea use in those with and without B12-deficiency.

Conclusions: Approximately a quarter of children with SCD demonstrated evidence of B12-deficiency, which is higher than expected. Cobalamin deficiency is associated with a constellation of clinically relevant symptoms that may be overlooked in patients with SCD. In addition, these patients may be uniquely at risk for adverse neurological sequelae when receiving treatment with nitrous oxide gas. B12-deficiency is easily corrected with an intramuscular injection of methylcobalamin. Although further study in a larger cohort is needed, screening for B12-deficiency may be warranted in patients with SCD.
C-5. Predictors and Outcomes of Immunoglobulin Supplementation in Children with B-Cell Acute Lymphoblastic Leukemia

Authors: Edington, Holly; DeGroote, Nicholas; Miller, Tamara; Chandrakasan, Shanmuganathan; Stevenson, Jason; Mertens, Ann; and Castellino, Sharon

Presenting Author: Holly Edington, MD
Type: Clinical - Poster

Background: Children with B-cell acute lymphoblastic leukemia (B-ALL) experience severe infections during treatment, which intravenous immunoglobulin (IVIG) supplementation may mitigate. IVIG supplementation currently occurs in approximately 30% of children with B-ALL, but evidence for its indications and benefits is sparse.

Objective: To compare disease and demographic characteristics by receipt of IVIG amongst children with B-ALL, and to evaluate outcomes following IVIG supplementation.

Methods: A retrospective cohort analysis examined children age 1-21 years with B-ALL treated at Children’s Healthcare of Atlanta from 2010 to 2017. Demographic, disease, treatment, and outcome data were collected from the electronic medical record. Patient characteristics were compared between patients with an immunoglobulin G (IgG) level checked vs. not checked, and by IVIG receipt among those with an IgG level checked. Multivariable logistic regression models identified factors associated with IVIG receipt. For IVIG recipients, general estimating equation modeling with Poisson distribution was used to compare rates of outcomes between IVIG supplemented and non-supplemented days.

Results: In total, 373 patients met inclusion criteria. IVIG was administered to 114 (30.5%) patients. An IgG level was checked in 251 (67.3%) patients. Median IgG nadir was lower for IVIG recipients vs non-recipients (404 vs 675mg/dL, p<0.01). IVIG recipients were younger at diagnosis (4 vs 6 years, p<0.01) and had more severe infections per 1,000 treatment days (4.2 vs 2.5, p<0.01). The odds of IVIG administration were lower for Non-White patients (Odds ratio (OR) 0.43, 95% Confidence interval (CI) 0.22-0.83), higher for patients with more than 2 severe infections during treatment (OR 2.57, 95% CI 1.28-5.18) and higher for National Cancer Institute standard risk patients with IgG nadir <500mg/dL (OR 7.45, 95% CI 3.54-15.70), adjusting for covariates. Rates of emergency department (ED) visits, hospitalization days, febrile neutropenia episodes and severe infections were lower during IVIG supplemented days vs. non-supplemented days (Rate ratio (RR) 0.52, CI [0.42-0.63]; RR 0.35, CI [0.26-0.46]; RR 0.29, CI [0.19-0.43]; RR 0.37, CI [0.27-0.49], respectively).

Conclusion: Patient characteristics differed by IVIG receipt status. IVIG supplementation can be beneficial in children with B-ALL to reduce infection-related outcomes. Prospective studies can help establish guidelines for IVIG supplementation and IgG monitoring.
C-6. Establishing Conditioned Reinforcers for Minimally Verbal Children with Autism Spectrum Disorder

Authors: Gerencser, Kristina; and Yosick, Rachel

Presenting Authors: Kristina Gerencser, PhD, BCBA-D
Rachel Yosick, PhD

Type: Clinical - Poster

BACKGROUND: Limited research and clinical guidance are available for children with Autism Spectrum Disorder (ASD) who are minimally verbal. While the research is growing, there remains limited clinical guidance. Many of these children have limited or restricted interests which for children with ASD often manifests as few items with which they engage with in a leisure context. When children have few leisure items that function as reinforcers during intervention, progress can be limited. Through conditioned reinforcement procedures clinicians can increase a child’s pool of reinforcers through pairing the delivery of known reinforcers with neutral stimuli to establish new, conditioned reinforcers. However, limited guidance on how to translation basic research into the clinical practice is available.

METHOD: Through a series of conditioning procedures new stimuli can be conditioned to create new reinforcers. One way is to use an operant discrimination training procedure to condition non-preferred leisure items as reinforcers (Taylor-Santa et al., 2014). Neutral tangible items were established as discriminative stimuli by reinforcing a specific engagement response in the presence of the item. Free-operant preference assessment probes of item engagement were conducted prior to conditioning, during conditioning, and post-conditioning. Following an observed treatment effect during free-operant probes, the conditioned item was tested for reinforcing efficacy while teaching a new skill. If a change in engagement was not observed during the free-operant probes, operant discrimination training procedure was discontinued, and a stimulus-stimulus conditioning procedure was implemented.

RESULTS: Results indicated that operant discrimination training for some children led to a greater level of engagement during free-operant probes. During the test for reinforcing efficacy, the conditioned item demonstrated some reinforcing properties, though not as strong as the participants’ primary reinforcers. For children with no engagement change in the free operant probe, increased engagement was observed during the stimulus-stimulus conditioning procedure.

CONCLUSION: Preliminary data are promising that through adaptive clinical conditioning procedures clinicians can establish new, conditioned reinforcers. Additional replications are needed.
C-7. ExaminingIntersectional and HIV-related Stigma Among Youth Living with HIV in Atlanta, Georgia

Authors: Goldstein, Madeleine; Moore, Shamia; Mohamed, Munira; Byrd, Rosalind; Zanoni, Brian C.; Camacho-Gonzalez, Andres; and Hussen, Sophia A.

Presenting Author: Madeleine Goldstein, DO
Type: Clinical - Poster

Background: Approximately 21% of new HIV infections in the United States occur among youth ages 13 to 24. Youth living with HIV (YLH) face a multitude of social and physical challenges, leading to suboptimal rates of engagement in HIV care. HIV-related stigma has been identified as a barrier to engagement in care for YLH, and is also associated with higher rates of depression, anxiety, and poorer quality of life. We conducted a qualitative study to examine experiences of stigma among YLH, as well as its possible influences on healthcare engagement.

Methods: We conducted 20 qualitative in-depth interviews among YLH who had recently transitioned to adult-oriented care within a large, comprehensive HIV care center in Atlanta, Georgia. Participants reflected on their experiences with stigma in pediatric care and during their healthcare transition. Thematic analysis contextualized how youth experienced enacted, anticipated, and internalized HIV and intersectional stigma (e.g., stigma related to racial and/or sexual minority identity) in both healthcare and non-healthcare settings.

Results: Sixteen (80%) participants were male, 17 (85%) acquired HIV horizontally, and the average age of participants was 28.5 years (SD 0.4 years). Most participants identified as Black/African American (19/95%) and gay (15/75%). Participants described stigma at intrapersonal, interpersonal, clinic, and community levels. Intrapersonal stigma was associated with delayed care seeking, isolation, and fear of disclosure. Stigma at the interpersonal level included discrimination from family and friends and avoidance of close relationships. At the clinic level, stigma included negative experiences with clinic peers and staff, which contributed to decreased engagement in care. Stigma in the community included differential treatment within non-HIV healthcare settings and was associated with feelings of helplessness related to current societal inequalities. Coping mechanisms for stigma included eliciting support from pediatric clinic providers and peers.

Discussion: Our findings show multiple intersecting stigmas contribute to barriers to healthcare at multiple levels for YLH, which has the potential to exacerbate existing health and social disparities. In order to improve engagement in care among YLH, future interventions should address the different mechanisms of stigma at community, clinic, and individual levels.
C-8. Feasibility of the Virtual Reality (VR) based Pediatric Display Enhanced Testing for Cognitive Impairment (PeDETECT) and mild Traumatic Brain Injury (TBI) tool to assess concussion in the pediatric emergency department (ED)

Authors: Grell, Robert; LaPlaca, Michelle; Wright, David; Simon, Harold K.; Yu, Austin; Hurley, Dylan; Murthy, Naina; Kulkarni, Megha; Santos, Justin; Sarnaik, Avnee; Waldon, Emma; Zafar, Farzina; Egbosiuba, Maureen; and Morris, Claudia R.

Presenting Author: Robert Grell, MD
Type: Clinical - Poster

Background: TBI is characterized by an external force to the head that results in an impairment of brain structure and/or function. The clinical presentation of a concussed patient is highly variable, resulting in up to 60% of children who meet the clinical criteria for concussion not receiving the diagnosis in the ED. Currently, there are no objective laboratory or imaging modalities that reliably diagnose concussion and clinical assessments are limited by subjectivity. VR is emerging as an objective method to measure the physical, cognitive, and behavioral impairments commonly associated with concussed patients. The PeDETECT system is a comprehensive multimodal tool that leverages VR to detect mild TBI in pediatric patients. Using a portable VR headset, the system assesses neuropsychological, balance, and oculomotor impairment using a series of tests that are gamified specifically for the pediatric population.

Objective: To assess the feasibility of the PeDETECT VR device in assessing pediatric mild TBI in the pediatric ED setting.

Methods: A prospective feasibility study comparing the percentage of patients in the pediatric ED presenting with head-injury who are able to complete the PeDETECT VR test vs completion in control ED subjects without TBI. Feasibility is defined by ≥80% completion. Secondary outcomes included user subjective feedback on the device, complex choice reaction time, working memory recall accuracy, and ED length of stay. Mean ± SD, unpaired Student t-test, and chi-square were used for statistical analysis when appropriate.

Results: To date, 73 patients have enrolled (15 head injury, 58 Controls). Mean age of the group was 12±2 years and 56% were male; no differences in age or gender were noted between cases and controls. Rate of completion was 88% overall with no difference between head injury vs. control groups (93% vs 86%, p=0.45 respectively). Male (n=41) vs female (n=32) rate of completion was 95% vs 77%, p=0.03.

Conclusions: These data demonstrate the feasibility of PeDETECT in the pediatric ED setting. Headset use was not limited by head-injury; however, gender differences were identified for rate completion. As enrollment is ongoing, future analysis will include secondary outcomes and the headset’s ability to detect concussion.

Authors: Hu, Xin; Marchak, Jordan; and Ji, Xu

<table>
<thead>
<tr>
<th>Presenting Author:</th>
<th>Xu Ji, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type:</td>
<td>Clinical - Poster</td>
</tr>
</tbody>
</table>

Background: Caring for a child diagnosed with cancer may adversely affect mental health (MH) of parents. This study examined MH care utilization for parents of children diagnosed with cancer, as compared to parents of children without cancer.

Methods: We used MarketScan® Commercial Database to identify families with children receiving therapy for leukemia, lymphoma, central nervous system, bone, or gonadal cancers (aged ≤21 years at diagnosis) in 2009-2017. We also identified families that had a child without cancer and were matched based on children’s age, gender, and region as controls. Outcomes included parents’ visits related to an MH disorder during the year after their child’s cancer diagnosis. Multivariable regressions were estimated to compare MH visits between 5,580 families with a pediatric cancer patient and 27,900 families in the control group, adjusting for sociodemographic factors and parental MH history.

Results: The proportions of families with ≥1 parent having visits related to anxiety (9.7% vs. 6.5%), depression (8.2% vs. 6.2%), or any MH disorders (17.3% vs. 13.4%) were higher in families that had a child with cancer than controls. These differences persisted in adjusted analyses. Families with cancer patients were 44%, 27%, and 24% (p-values<0.001) more likely than controls to have ≥1 parent experiencing anxiety-related, depression-related, and any MH visits, respectively. No difference was seen in substance use disorder related visits.

Among families with a cancer patient, parental MH history and families with a child who received hematopoietic stem cell transplantation (vs. surgery only) were more likely to have ≥1 parent experiencing MH visits (p-values<0.05). Having ≥3 children in household was associated with a lower likelihood of parental MH visits (p-values<0.05). When examining MH needs for fathers and mothers separately, the likelihoods of anxiety-related (8.0% vs. 4.0%), depression-related (7.0% vs. 3.2%), and any MH visits (13.8% vs. 8.1%) were higher among mothers than fathers.

Conclusions: Parents of pediatric cancer patients have higher needs for MH care compared with the general parent population. Our finding underlines the importance of interventions toward targeted MH counseling and supportive strategies for vulnerable parent groups, including mothers and those caring for children undergoing complex treatment plans.
C-10. Clinical and Serological Responses to COVID-19 Vaccination in Children with History of MIS-C

Authors: Perez, Maria A.; Hsiao, Hui-Mien; Lu, Austin; Hussaini, Laila; Macoy, Lisa; Chahroudi, Ann; Anderson, Evan J.; Rostad, Christina A.

Presenting Author: Kristy Rostad, MD
Type: Clinical - Poster

Background: The clinical and serological responses to COVID-19 vaccination in children with a history of multisystem inflammatory syndrome (MIS-C) compared to healthy controls are unknown.

Methods: We prospectively enrolled 3 hospitalized children with MIS-C and 6 healthy outpatient pediatric controls and measured SARS-CoV-2 spike receptor binding domain (RBD) and nucleocapsid (N) protein IgG antibodies by enzyme-linked immunosorbent assay longitudinally and pre- and post-BNT162b2 COVID-19 vaccination. We performed a questionnaire following each vaccine dose to evaluate for local and systemic reactogenicity and other adverse effects.

Results: During the acute hospitalization, children with MIS-C had peak SARS-CoV-2 RBD and N IgG titers of (3.75 log10, 95% CI 3.21 to 4.3) and (3.25 log10, 95% CI 3.19 to 3.31) respectively, which plateaued following discharge. Vaccination with two doses of BNT162b2 significantly increased mean RBD IgG titers by 1.52 log10 (95% CI 1.05 to 2.00 log10) (Figure). SARS-CoV-2 RBD IgG titers following the second dose of BNT162b2 were similar in children with history of MIS-C vs. vaccinated healthy pediatric controls (4.89 vs. 4.65 log10, P=0.893). Local and systemic reactogenicity and antipyretic utilization following both doses of BNT162b2 were similar among children with MIS-C vs. healthy controls. The most common solicited adverse effects were fatigue, headache, and local tenderness. One child with history of MIS-C reported mild headache, severe fatigue, back pain, and rash following BNT162b2 dose 2. While the rash self-resolved after 2 days, and the back pain resolved following a visit to the chiropractor, his reported mild headache and severe fatigue were persistent at 7-week follow-up.

Conclusions: COVID-19 vaccination of children with history of MIS-C significantly boosted SARS-CoV-2 RBD IgG titers to levels comparable to those of healthy vaccinated pediatric controls. Further study is needed to understand the local and systemic reactogenicity of COVID-19 vaccination in this population.
C-11. Comprehensive Genomic Profiling of High-Risk Pediatric Cancer Patients has a Measurable Impact on Clinical Care

Authors: Summers, Ryan J; Castellino, Sharon M; Porter, Christopher C; MacDonald, Tobey; Carter, Alexis; Pauly, Melinda G; Cash, Thomas; Mitchell, Sarah; Castellino, R Craig; Fangusaro, Jason; Pencheva, Bojana; Bhasin, Manoj; Basu, Gargi; Szelinger, Szabolcs; Wechsler, Dan; Graham, Douglas K; and Goldsmith, Kelly C

<table>
<thead>
<tr>
<th>Presenting Author:</th>
<th>Ryan Summers, MD, FAAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type:</td>
<td>Clinical - Poster</td>
</tr>
</tbody>
</table>

Advances in genomic sequencing technology have led to the rapid adoption of childhood tumor genomic profiling platforms ranging from targeted gene panels to whole exome sequencing. However, the most valuable approach to genomic profiling of pediatric cancers is yet to be defined. To assess the clinical impact of genomic profiling, we conducted a prospective precision medicine trial, employing whole-exome sequencing (WES) of tumor and germline tissue and whole-transcriptome sequencing (RNA Seq) of tumor tissue to characterize the mutational landscape of 127 tumors from 126 unique patients across the spectrum of pediatric brain tumors, hematologic malignancies, and extracranial solid tumors. We identified somatic tumor alterations in 121/127 (95.3%) tumor samples and identified cancer predisposition syndromes based on known pathogenic germline mutations in cancer predisposition genes in 9/126 patients (7%). Activating alterations in the targetable PI3K and MAPK signaling pathways were identified across all tumor groups, providing a rationale for tumor-agnostic trials of molecularly targeted therapies. Fifty gene fusions were identified, 6 of which were completely novel. Nine fusions found in 13 patients were potentially targetable. Additionally, we developed a novel scoring system for measuring the impact of tumor and germline sequencing. At least one impactful finding from the genomic results was identified in 108/127 (85%) samples sequenced. A recommendation to consider a targeted agent was provided for 82/126 (65.1%) patients. Twenty patients have received therapy with a molecularly targeted agent, representing 24% of patients who received a targeted therapy recommendation and 16% of the total cohort. Paired tumor/normal WES and tumor RNA Seq of de novo or relapsed/refractory tumors was feasible and clinically impactful in high-risk pediatric cancer patients.