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ABSTRACT BOOK

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EMORY UNIVERSITY

TABLE OF CONTENTS

ORAL PRESENTATION RESEARCH ABSTRACTS *(in the order of presentations)*

Basic Science and Translational Research

Clinical and Outcomes Research

MINI- (RAPID-FIRE) PRESENTATION RESEARCH ABSTRACTS *(in the order of presentations)*

Basic Science and Translational Research

TABLE OF CONTENTS

MINI- (RAPID-FIRE) PRESENTATION RESEARCH ABSTRACTS *(in the order of presentations)*

Basic Science and Translational Research *(continued)*

**Rapid-Fire Finalist

TABLE OF CONTENTS

MINI- (RAPID-FIRE) PRESENTATION RESEARCH ABSTRACTS *(in the order of presentations)*

Clinical and Outcomes Research

**Rapid-Fire Finalist

TABLE OF CONTENTS

POSTER SESSION RESEARCH ABSTRACTS BY THEME

Poster Presentations

TABLE OF CONTENTS

POSTER SESSION RESEARCH ABSTRACTS BY THEME *(continued)*

Poster Presentations

TABLE OF CONTENTS

POSTER SESSION RESEARCH ABSTRACTS BY THEME *(continued)*

Poster Presentations

Single-cell Transcriptomic Analysis Reveals Neuropathogenic Mechanisms of Brain Injury Following Infant Postnatal Zika Virus Infection

Authors: Edara, Venkata-Viswanadh; Schoof, Nils; Burgess, Divine; Richardson, Rebecca; Freeman, Sienna; Sampson, Maureen; Moore, Kathryn; Suthar, Mehul; Bosinger, Steven; Raper, Jessica; Sloan, Steve; and Chahroudi, Ann

Presenting Author: Venkata Viswanadh Edara, PhD

Background: Consequences of postnatal ZIKV infection in infants and children are not well understood. We have previously shown abnormal brain structure and function that is predictive of behavior in infant rhesus macaques infected with ZIKV postnatally. Here, we explored the brain regions, cells, and pathways impacted by postnatal ZIKV infection to suggest mechanisms of injury and neuropathogenesis.

Methods: Infant rhesus macaques (RMs) were infected with ZIKV at one month of age and euthanized 14 days after infection for single cell transcriptomic analyses of the hippocampus, amygdala, and striatum. ZIKV-infected infant RMs were compared to age and sex-matched uninfected controls. Bioinformatic approaches using R (V4) and Seurat (V4) were utilized and, after quality control, 105,421 cells from controls and 94,975 cells from ZIKV-infected animals were analyzed.

Results: Acute postnatal ZIKV infection induced a subset of activated microglia characterized by upregulation of transcription factors involved in interferon signaling, leading to increased expression of interferon-stimulated genes (ISGs), including IFI6, IFI27, and MX1. In mature oligodendrocytes, ZIKV infection significantly downregulated genes associated with ATP metabolism, sterol biosynthesis, and oligodendrocyte differentiation. Gene set enrichment analysis revealed a significant negative enrichment of a set of 147 autism spectrum disorder (ASD)-associated genes. Behaviorally, ZIKV-infected infant rhesus macaques exhibited decreased orientation responses and increased temperament reactivity, suggesting early neurodevelopmental disruption.

Conclusions: Our results show that acute postnatal ZIKV infection leads to CNS immune activation, and disruption of metabolic and differentiation pathways in oligodendrocytes. The associated downregulation of ASD-linked genes and observed behavioral alterations suggest that postnatal ZIKV exposure may contribute to long-term deficits in social interactions. These results highlight the potential for early-life ZIKV infection to alter brain development through both molecular and behavioral pathways.

Tumor Microenvironment-mediated Immune Evasion by the Immune Checkpoint Siglec-15 In Hematologic Malignancies

Authors: Francis, Dailia B; Dougan, Jodi; Michaud, Marina; Bhasin, Manoj; and Porter, Christopher

Presenting Author: Dailia Francis, MD, PhD

Background: Primary refractory and relapsed (r/r) hematologic malignancies remain a challenge in pediatric patients. While hematopoietic stem cell transplant and novel immunotherapies such as immune checkpoint inhibitors and chimeric antigen receptor (CAR) T-cell therapy have shown some promise in this population of patients, outcomes in r/r remain dismal for many patients. This highlights the urgency to identify factors that drive refractory and/or relapsed disease that will address current therapeutic gaps and improve overall patient survival.

Siglec-15 (Sig-15), an immunoglobulin-like lectin, is a critical immune suppressor that is highly expressed in various human cancers and intra-tumoral myeloid cells. Importantly, inhibiting Siglec-15, either through genetic knockout or knockdown, had a restorative effect on local anti-tumor immune responses and abrogated tumor progression. While reported in solid malignancies, a role for Siglec-15 in promoting disease progression in hematologic malignancies has not yet been described.

Methods: We have evaluated Sig-15 expression in primary human lymphoma patient samples as well as various lymphoma (human and mouse) cell lines using western blot, quantitative PCR as well immunohistochemistry and immunofluorescence methods. Sig-15 expression was inhibited through genetic downregulation in the well-established murine lymphoma cell line A20 or the leukemia cell line BAML and injected into immune competent and immune deficient mice. Analysis of the immune microenvironment was performed using single cell RNA (ScRNA) sequencing as well as multiparameter flow cytometry was on bone marrow, spleen and lymph nodes harvested at various time points.

Results and Conclusions: Sig-15 is highly expressed in lymphoma and leukemia cell lines as well as primary patient samples. Disease progression in immune competent but not immunodeficient recipients, consistent with a role for Sig-15 in immune evasion in lymphoma. Our data demonstrates that there are Sig-15 dependent changes in the bone marrow and spleen by scRNA sequencing. Further, preliminary analysis by flow cytometry reveals significant changes in the immune microenvironment that suggests Sig-15 may promote aberrant myelopoiesis in murine models of B-ALL and lymphoma. Together, these data implicate Sig-15 as an immune checkpoint that may be inhibited therapeutically to promote an immune response to lymphoma cells.

miR-21/METTL3 Axis Regulates CFTR Expression in CF Lung Disease

Authors: Kang, Bum-Yong; Yi, Erin; Ozuna, Hazel; Miralda, Irina; Durfey, Samantha; Lee, Chris; Murphy, Ashley; Shrestha, Mahesh; Moran, John; and Kopp, Benjamin

Presenting Author: Bum-Yong Kang, PhD

Background: Cystic fibrosis (CF) results from mutations in the CF transmembrane conductance regulator (CFTR) gene, leading to pulmonary vascular impairments and progressive lung damage, particularly in aging populations. Emerging studies suggest that N6-methyladenosine (m6A) epitranscriptomic modification regulates RNA processing and metabolism, influencing downstream biological effects. Additionally, microRNAs (miRNAs) play key roles in pulmonary disease and regulate m6A-modifying factors. However, the molecular mechanisms by which the miRNA-m6A epitranscriptomic modification axis regulates CFTR expression remain unclear. We hypothesize that reduced CFTR levels increase miRNAs, which inhibits m6A modification, thereby impairing CFTR mRNA translation.

Methods: We reanalyzed our previous study using blood transcriptomic data from non-CF individuals and people with CF (pwCF, n=20 each, average age 22 years) to identify differentially expressed m6A modification factors (writers, erasers, and readers) and miRNAs. To validate our findings, we performed qRT-PCR on lung tissues from non-CF and pwCF. To determine whether CFTR depletion affects miR-21 and METTL3 levels, human umbilical vein endothelial cells (HUVECs) were transfected with scrambled (SCR) or CFTR-targeting siRNA. Additionally, HUVECs were transfected with SCR, miR-21 mimics, or METTL3-targeting siRNA to dissect the molecular mechanisms of miR-21-METTL3-CFTR circuit.

Results: Epitranscriptomic analysis revealed that m6A modification factors (writers, erasers, and readers) and miRNAs were differentially expressed between pwCF and non-CF groups. Notably, METTL3 (writer) was downregulated, whereas YTHDF3 (reader) was upregulated in pwCF. We confirmed that METTL3 was decreased in pwCF lungs and in siCFTR-treated HUVECs. Among the screened METTL3-targeting miRNAs, miR-21 was significantly upregulated in pwCF. In silico analysis (TargetScan) predicted miR-21 binding sites in METTL3's 3'UTR, confirmed by increased miR-21 in pwCF lungs and siCFTR-treated HUVECs. miR-21 overexpression (miR-21 mimic) reduced both METTL3 and CFTR levels in HUVECs. In silico analysis (SRAMP) identified nine potential m6A binding sites in CFTR. Finally, siRNA-mediated METTL3 depletion decreased CFTR expression in HUVECs, supporting its role in CFTR regulation.

Conclusion: Our findings identify a novel CFTR-miR-21-METTL3 regulatory circuit in CF pathogenesis, providing new insights into the roles of miRNAs and m6A modification in CF lung disease.

YAP Suppresses HRK Through the PRC2 Complex Leading to Neuroblastoma Cell Survival

Authors: Shim, Jenny; Hernandez, Pamela; Sewani, Soha; McCraw, Morgan; and Goldsmith, Kelly

Presenting Author: Jenny Shim, MD

Background: Gene expression analyses showed a significant decrease in expression of genes suppressed by the Yes-Associated Protein (YAP) in relapsed neuroblastoma (NB), suggesting increased YAP-mediated transcriptional repression at relapse. YAP is a transcriptional cofactor that binds TEAD family transcription factors to regulate target gene expression. Previously we showed that YAP promotes chemotherapy and MEK inhibitor resistance in RAS-mutated NBs in vivo by suppressing Harakiri (HRK), a BH3-only pro-apoptotic protein activated by tumor environmental stress.

Aims: Our objective is to elucidate the mechanism through which YAP suppresses key target genes and to identify YAP-directed therapies to restore therapy response in NB.

Methods: We interrogated publicly available ATAC-seq/ChIP-seq datasets of NB. We performed co-immunoprecipitation to identify protein-protein interactions of YAP and EZH2 in SK-N-AS and NLF. We treated YAP-expressing and YAP-null NBs with EZH2 inhibitor tazemetostat and evaluated gene expression. We tested IAG933, a novel YAP-TEAD small molecule inhibitor alone and in combination with MEK inhibitor trametinib in RAS-mutated NBs or with EZH2 inhibitor tazemetostat.

Results: ATAC-seq/ChIP-seq datasets showed increased H3K27me3 occupancy and decreased chromatin accessibility in the promoter region of HRK in YAP-expressing NBs. YAP immunoprecipitation confirmed TEAD2, as well as EZH2 (catalytic subunit of the PRC2 complex) binding to YAP in RAS-mutated NB. Inhibition of EZH2 with tazemetostat in YAP-expressing NBs (SK-N-AS, NLF, IMR5) led to upregulation of HRK compared to vehicle, while tazemetostat treatment of YAP-null NGP did not show changes in HRK expression. IAG933 treatment of SK-N-AS and NLF led to downregulation of CTGF, CYR61, and upregulation of HRK. IAG933 in combination with trametinib or tazemetostat exhibited synergistic cytotoxicity against RAS-mutated NBs.

Conclusions: YAP recruits key PRC2 complex member EZH2 to mediate YAP-TEAD downstream gene suppression in NB. Treatment with novel YAP-TEAD inhibitor augments MEK inhibitor and EZH2 inhibitor responses in NB. Further studies with IAG933 in combination with other broad targeted therapies are ongoing in relapsed NB models.

Genetic Variants Associated with Factor V Deficiency May Affect FV-short Splicing

Authors: Zimowski, Karen L; Patel, Seema R; Meeks, Shannon L; and Doering, Christopher B

Presenting Author: Karen Zimowski, MD

Background: Severe factor V (FV) deficiency is a rare inherited bleeding disorder associated with mild symptoms. This mild phenotype occurs due to lowered levels of tissue factor pathway inhibitor (TFPI), an intrinsic anticoagulant that interacts with FV. Unexpectedly, a Dutch study of patients with ultra-rare bleeding disorders reported the highest bleeding scores for patients with FV deficiency. Rather than causing FV deficiency, certain genetic variants in the FV gene (F5), including the F5-Atlanta deletion (F5-ATL), enhance the removal of an exon-like intron ("exitron") from F5 exon 13 (F5E13), producing an alternative FV isoform, FV-short. FV-short binds, stabilizes, and increases TFPI in plasma, resulting in bleeding. We hypothesized that some variants associated with FV-deficiency may impact FV-short splicing.

Methods: The European Association for Haemophilia and Allied Disorders F5 Gene Variant Database was queried to identify F5E13 variants residing within the region of F5-ATL. Selected variants were cloned into a green fluorescence protein (GFP) based reporter, stably transfected into FLP-IN HEK293T cells, and analyzed for alternative splicing using flow cytometry. Results were compared to reporter constructs incorporating the wild-type (WT) F5 exitron, the F5 exitron harboring F5-ATL, and GFP cDNA alone.

Results: Four variants associated with FV deficiency (FV<1%) were selected for functional splicing analysis: c2615delG, c.2862delT, c.3153_3154insCT, and c.3037C>T. The first 3 cause a terminal frameshift whereas the last is a nonsense variant. Significant differences were detected in median fluorescence intensity (MFI) from GFP+ live cells between the variants (Kruskal-Wallis $p<0.0001$). Higher MFI was seen in cells transfected with c.3153_3154insCT compared to those using WT-F5 (Dunn's $p = 0.0250$); this was not significantly different than the GFP and F5-ATL controls. Cells transfected with reporter constructs containing c.2615delG and c.2862delT demonstrated MFI comparable to that of WT-F5. There was a non-significant trend towards higher MFI in cells harboring c.3037C>T compared to WT-F5.

Conclusions: Using a reductionist model, our study suggests F5 c.3153_3154insCT may impact FV-short splicing. Further studies are needed to confirm this finding and to determine its impact on TFPI and bleeding. Such studies could improve genotype - phenotype correlation in FV deficiency and improve clinical management of these patients.

Complement Dysregulation During Vaso-Occlusive Episodes Can Predict Development of Acute Chest Syndrome in Pediatric Sickle Cell Disease

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Presenting Author: Akshaya Arjunan, MD

Background: Vaso-occlusive episodes (VOE) remain the leading cause of hospitalizations and morbidity in pediatric sickle cell disease (SCD) yet, supportive care with analgesia and blood transfusions are the mainstay of treatment. Complement dysregulation is implicated in hemolysis and organ damage in SCD, however few studies have evaluated its role during VOE and acute chest syndrome (ACS). We hypothesize that complement dysregulation is a key driver in the pathophysiology of VOE and ACS and can serve as a novel biomarker and therapeutic target.

Methods: This single institution, prospective study enrolled pediatric SCD patients admitted for VOE at the Children's Healthcare of Atlanta from 2018-2021. Blood samples were collected within 48 hours of admission (acute) and at clinic follow up (steady-state) ≥4 weeks later. Clinical history and hemolytic biomarkers were collected. Levels of C3, C3a, C4, C4a, C4d, C5, C5a, C5b-9, and Bb were measured using commercial ELISA kits. Change in hemolysis and complement activation from acute to steady-state and in patients who developed ACS was analyzed with paired and unpaired statistical analyses.

Results: Seventy patients were enrolled accounting for 100 VOE and 69 steady-state episodes. In paired data samples, a significant decrease in mean hemoglobin (Hgb, n=60, p<0.0001) was seen between steady-state (mean=8.89g/dL) and acute (mean=7.93g/dL) while lactate dehydrogenase (LDH, n=52, p=0.0222) increased (mean=465.6 vs. 568.1 U/L). In paired plasma samples (n=30), a significant increase from steady-state to acute was seen in C4d (lectin pathway: median= 0.20 vs. 0.29 mg/L, p=0.0030), Bb (alternative: median= 1.35 vs. 1.96 ug/mL, p<0.0001), C3a & C5a (anaphylatoxins: p=0.0004 & p=0.0026) and sC5b-9 (terminal: p=0.0277). Complement levels at enrollment demonstrated good discriminatory ability for predicting the development of acute chest syndrome for C4d (AUC 0.75, p=0.0061), Bb (AUC 0.71, p=0.0210), C3a (AUC=0.77, p=0.0027), and C5a (AUC 0.72, p=0.0135).

Conclusion: We found evidence of proximal (lectin and alternative) complement pathway activation during VOE in a large pediatric SCD cohort. This was further pronounced in patients who developed ACS suggesting that complement and LDH could serve as novel biomarkers in identifying high-risk patients. Proximal complement pathway targeting may be an interesting new VOE treatment that could prevent ACS in SCD.

PFAS and Altered Glucose Homeostasis in Adolescents Following Bariatric Surgery

Authors: Baumert, Brittney O.; Costello, Elizabeth; Li, Zhenjiang; Midya, Vishal; Pan, Shudi; Ryder, Justin; Inge, Thomas; Jenkins, Todd; Sisley, Stephanie; Xanthakos, Stavra A.; Walker, Douglas I.; Stratakis, Nikos; Valvi, Damaskini; Bartell, Scott M.; Slitt, Angela L.; Kohli, Rohit; Rock, Sarah; La Merrill, Michele A.; Eckel, Sandrah P.; Aung, Max T.; McConnell, Rob; Conti, David V.; and Chatzi, Lida

Presenting Author: Brittney Baumert, PhD, MPH

Background: As obesity and metabolic disease have become more prevalent in youth in the US, bariatric surgery has emerged as an effective treatment for obesity and type 2 diabetes, when comorbid with severe obesity. Per- and polyfluoroalkyl substances (PFAS) have been linked to dysregulated glucose metabolism; however, this relationship has not been investigated following a large weight-loss intervention.

Methods: Adolescents (n=186) enrolled in the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS) study who underwent bariatric surgery between 2007-2012 were included. PFAS (PFHxS, PFHpA, PFHpS, PFOA, PFOS, PFNA, PFDA, PFUnDA) were measured in plasma at baseline. Linear and logistic regression were used to examine cross-sectional associations between log2-transformed PFAS (ng/mL) and glucose (mg/dL), insulin (uIU/ml), HbA1c (%), and HOMA-IR. Linear mixed models were used to examine the longitudinal associations between PFAS and all outcomes. PFAS mixtures effects at each visit were assessed using quantile g-computation. All models adjusted for age, sex, race, parents' income, study site, and use of diabetes medication.

Results: PFHxS was positively associated with greater increases in fasting glucose in the one-to-five year post-operative period: a PFHxS level of 1.95 log2-ng/mL was associated with a 3.30mg/dL (95%CI: 1.23-5.37) increase over four years, while a PFHxS level of -0.16 log2-ng/mL was associated with a 1.19 mg/dL (95%CI: -0.91, 3.29) increase. PFHxS, PFHpS, and PFHpA were positively associated with fasting insulin and HOMA-IR at baseline, but no association was observed in the one-to-five-year post-operative period. Each quartile increase in the PFAS mixture was associated with higher fasting insulin and HOMA-IR at the baseline visit, but this association did not persist at follow up visits.

Conclusions: PFHxS exposure may attenuate improvement in fasting glucose levels after bariatric surgery. PFAS exposure is associated with insulin sensitivity prior to surgery but does not appear to have any effect on improvements in fasting insulin or HOMA-IR in the years following surgery.

The Neighborhood Exposome and Persistently Distressing Psychotic-like Experiences

Authors: Ku, Benson; Chen, Yinxian; Yuan, Qingyue; Christensen, Grace; Dimitrov, Lina; Risk, Benjamin; and Huels, Anke

Presenting Author: Benson Ku, MD, PhD

Background: Recent research has demonstrated that domains of social determinants of health (SDOH) (e.g. air pollution and social context) are associated with psychosis. However, SDOHs have often been studied in isolation. This study investigated distinct exposure profiles, estimated their associations with persistent distressing psychotic-like experiences (PLE), and evaluated whether involvement in physical activity partially explains this association.

Methods: Analyses included 8,145 young adolescents from the Adolescent Brain and Cognitive Development Study. Data from the baseline and three follow-ups were included. Multi-dimensional neighborhood-level exposures were used to form a neighborhood exposome (NE) score. Area-level geocoded variables spanning various domains of SDOH, including socioeconomic status, education, crime, built environment, social context, and crime, were also clustered using a self-organizing map method to identify exposure profiles. Genetic risk was measured by a multi-ancestry schizophrenia polygenic risk score (SCZ-PRS). Generalized linear mixed modeling tested the associations between exposure profiles, and persistent distressing PLE and physical activities (i.e. team and individual sports), adjusting for individual-level covariates including age, sex, race/ethnicity, highest level of parent education, family-relatedness, and study sites.

Results: Five exposure profiles were identified. Compared to the reference Profile 1 (suburban affluent areas), Profile 3 (rural areas with low walkability and high ozone), and Profile 4 (urban areas with high SES deprivation, high crime, and high pollution) were associated with greater persistent distressing PLE. Team sports mediated 6.14% of the association for Profile 3. The association between NE score and persistent distressing PLE was statistically significantly attenuated as SCZ-PRS increased (OR for interaction = 0.92, 95% CI: 0.86, 1.00, P = 0.039).

Conclusions: This study found that neighborhoods characterized by rural areas with low walkability and urban areas with high socioeconomic deprivation, pollution concentrations, and crime were associated with persistent distressing PLE. Findings suggest that various social-environmental factors may differentially impact the development of psychosis, particularly among those with lower genetic risk.

Integrin α_4 and CCR5 on IFNγ-activated MSC Determine Biodistribution to Critical Sites for aGVHD Prophylaxis in Mice

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Presenting Author: Elisabetta Manuela Foppiani, PhD

Poster Number: 41

Background: Mesenchymal stromal cells (MSC) activated with interferon-γ (γMSC) exhibit potent immunosuppressive properties, yet nonactivated MSC remain the focus of clinical trials. Extensive mouse studies show that γMSC can prevent acute Graft versus Host Disease (GVHD) after hematopoietic cell transplantation, but the molecular mechanisms affecting their distribution post-IV infusion and the sites of activity remain unclear. This study aims to identify the site(s) of activity and elucidate the molecular and cellular mechanisms governing γMSC trafficking.

Methods: A mouse model of fully MHC mismatched hematopoietic cell transplantation (HCT) was used. Human γMSC were infused 24 hours post-HCT, and their tissue localization was assessed. CRISPR/Cas9 was used to modify γMSC, and in vitro analyses conducted using IncuCyte or fluorescence microscopy. A chemokine array was used to see the chemokines involve in the distribution. Macrophages depletion with clodronate was performed to study their role in trafficking and GVHD prevention.

Results: We observed that γMSC were enriched in gut-associated lymphoid tissue (GALT) 16 hours post-IV infusion, critical site for the development of aGVHD. In vitro, γMSC exhibited significantly greater adhesion to spleen endothelial cells compared to other SLO endothelial cells, and integrin α4 was crucial for this interaction, as α4 knockdown reduced binding by 94%. We identified CCL3, CCL4, and CCL5 as key chemokines upregulated in activated T cell CM, and blocking these chemokines collectively abolished γMSC migration. γMSC expressed CCR1 and CCR5, with CCR5 being upregulated upon exposure to activated T cell CM, and CCR5 knockdown in γMSC reduced migration by 90%. In vivo, knockdown of α4 or CCR5 in γMSC impaired their trafficking to GALT and signicantly the prophylactic effects against GVHD, although T cell suppression by γMSC was retained. Furthermore, depletion of monocytes/macrophages by liposomal clodronate did not affect γMSC trafficking to the spleen but abolished the GVHD-suppressive activity of γMSC, indicating that macrophages are not required for γMSC trafficking, but essential for their therapeutic function.

Conclusion: Our study reveals that the coordinated expression of integrin α4 and CCR5 on γMSC governs their trafficking to GALT, where they exert their immunosuppressive effect and significantly reduce GVHD in a mouse model. These findings highlight the importance of GALT localization in γMSC efficacy, offering insights that could inform strategies to enhance the therapeutic potential of γMSC in GVHD prevention.

Defining and Targeting Gamma Delta (γδ) T Cell Therapy-induced Apoptosis in Neuroblastoma

Authors: Hudson, Zuri; McCraw, Morgan; and Goldsmith, Kelly

Presenting Author: Zuri Hudson, DO

Poster Number: 2

BACKGROUND: High risk neuroblastoma (NB) is a pediatric solid tumor with an event free survival <50%. Gamma delta (γδ) T cells are an adoptive cell therapy (ACT) that are cytotoxic to NB cells, lack alloreactivity, and can differentiate normal from malignant cells. They induce apoptosis via three potential mechanisms: I) FAS death receptor-mediated Type I apoptosis, II) FAS mediated Type II apoptosis, or III) release of perforin/granzyme. NBs dysregulate apoptosis pathways with downregulation of Caspase 8 via hypermethylation or upregulation of mitochondrial pro-survival Bcl-2 proteins. We hypothesize that NBs capitalize on altered apoptotic machinery to resist γδ T cell-induced apoptosis, and by therapeutically restoring apoptosis sensitivity with targeted agents, we will enhance γδ T cell therapy potency against HR NB.

DESIGN/METHODS: Western blot in γδ T cell sensitive and resistant NB models characterized Caspase 8, CD95/FAS, BAK/BAX, BID and Bcl-2 family protein expression. We will utilize CRISPR/CAS9 to knock out key apoptosis players then perform co-culture assays with γδ T cells, anti-GD2 antibody, dinutuximab, and Bcl-2 inhibitor, venetoclax, measuring a downstream apoptosis product, cleaved caspase 3 (CC3). We will determine whether treatment with venetoclax or hypomethylating agent, decitabine, can enhance γδ T cell induced tumor death.

RESULTS: Western blot has demonstrated different levels of Caspase 8, FAS/CD95, and BID/Bcl-2 family expression in various NB models. Coupling this data with GD2 expression by flow cytometry, GD2 and Bcl-2 expressing NB cell line CHLA-15 has been utilized for co-culture assays. Venetoclax treatment significantly increased NB apoptosis sensitivity in response to γδ T cells with and without dinutuximab at 48 hours of incubation with CHLA-15.

CONCLUSIONS: As the killing mechanism of γδ T cells is largely unknown, my goal is to identify which of the pathways in NB is utilized for γδ T cell-induced apoptosis by understanding the type of apoptosis that is occurring in sensitive cell lines and why apoptosis does not occur in resistant cell lines. Apoptotic resistance mechanisms such as upregulation of Bcl-2 proteins, can be overcome in a Bcl-2 dependent NB cell line. The work accomplished will strengthen clinical efforts enhancing trials utilizing γδ T cells for high risk and relapsed/refractory NB.

Engineered Humanized L-Asparaginase Variants Demonstrate Potent Antileukemic Activity and Attenuated Immunogenicity

Authors: Knight, Kristopher; Brown, Harrison; Karpen, Matthew; Spencer, H. Trent; Doering, Christopher; and Raikar, Sunil

Presenting Author: Kristopher Knight, BS

Poster Number: 3

Background: L-asparaginase (L-ASNase) is a critical component of the frontline therapy for acute lymphoblastic leukemia (ALL), but its clinical utility is limited by high immunogenicity. Current clinical L-ASNases, are derived from Escherichia coli or Erwinia chrysanthemi, exhibiting only ~30% sequence identity with human L-ASNase, leading to hypersensitivity reactions in up to 30% of patients. These immune responses often require early treatment discontinuation, compromising disease-free survival. To address this limitation, we applied ancestral sequence reconstruction (ASR), an evolutionary protein engineering approach to generate novel humanized L-ASNase protein drug candidates. Guinea pig L-ASNase, sharing ~70% identity with the human ortholog and possessing favorable enzymatic and cytotoxic properties, was used as the evolutionary anchor.

Method: Ten An-L-ASNase variants, spanning the phylogenetic lineage between guinea pig and human, were computationally reconstructed, synthesized, and expressed. Enzymatic activity was measured using a modified Nessler's assay, and antileukemic efficacy was assessed in CCRF-CEM and MOLT-4 cell lines via MTT assays. Immunogenicity was profiled in silico using HLA alleles with high global prevalence and predicted high affinity binding to ASNase epitopes. Linear B-cell epitope prediction was also performed to evaluate potential antibody generation. To further reduce immunogenicity, hybrid ancestral-human L-ASNases were engineered by replacing the ancestral C-terminal ankyrin domain with the corresponding human sequence.

Results: Among the An-L-ASNases, An-104 and An-107 demonstrated enzymatic activity and cytotoxicity comparable to E. coli L-ASNase, with similar IC50 values against both cell lines. After accounting for human epitopes, all An-L-ASNases were predicted to have significantly reduced T-cell and B-cell responses with respect to allergenicity and immunogenicity risk compared to bacterial L-ASNases. Hybrid ancestral-human proteins incorporating the non-functional human C-terminal domain achieved 90–99% sequence identity to human L-ASNase and are currently being characterized.

Conclusion: This study highlights ASR as a robust platform for bioengineering next-generation L-ASNase therapeutics. An-L-ASNase variants such as An-104 and An-107 retain potent antileukemic activity while substantially lowering predicted immunogenicity risk. These findings pave the way for more durable treatment options by addressing a major clinical barrier to sustained L-ASNase therapy in ALL.

Parallel Ab-Seq and scRNA-Seq of Lung-Recruited Neutrophils in Human Cystic Fibrosis

Authors: Li, Zhuo; Moncada, Diego; Leese-Thompson, Collin; Luthra, Deepali; Dobosh, Brian; Aldeco, Mila; and Tirouvanziam, Rabindra

Presenting Author: Zhuo Li, MS

Poster Number: 22

Introduction: Cystic fibrosis (CF) lung disease has a profound airway neutrophil phenotype termed GRIM (Granule-Releasing, Immunomodulatory, and Metabolically active). GRIM neutrophils arise after naïve blood neutrophils transmigrate into the CF lung, undergoing a transcriptional burst, elevated CD63 and reduced CD16 surface expression, granule hyperexocytosis (e.g., elastase, myeloperoxidase, arginase-1), and a shift toward anabolic metabolism, coupled with impaired bacterial killing. This study investigates the roles of chromatin-modifying enzymes HDAC11 and EZH2 in GRIM phenotype development.

Methods: We used an in vitro transmigration model conditioned with extracellular vesicle (EV)-rich, cell-depleted CF airway supernatant (CFASN). Neutrophils were treated with CFASN alone or with HDAC11 (SIS17) or EZH2 (EPZ6438) inhibitors. Multi-omics approaches included bulk RNA-Seq, single-cell RNA-Seq (scRNA-Seq), antibody-based sequencing (Ab-Seq), single-nuclei ATAC-Seq, and untargeted EV proteomics. Flow cytometry of CF patient blood and sputum samples assessed surface markers (CD16, CD63, CD66b, PD-1, PD-L1, CSF1R, EGFR).

Results: scRNA-Seq and Ab-Seq revealed broad transcriptional activation and a CD16^low/CD63^high phenotype in CFASN-conditioned neutrophils. ATAC-Seq identified altered chromatin accessibility at immediate early genes (e.g., FOSL1, JUNB, FOSB, JDP2), suggesting epigenetic regulation. Inhibiting HDAC11 or EZH2 restored a CD16^high/CD63^low phenotype and bacterial killing capacity without reversing transcriptional activation, indicating post-transcriptional or post-translational mechanisms. EV proteomics showed HDAC11 inhibition altered vesicle protein cargo, implicating it in cell–cell signaling. Flow cytometry confirmed upregulation of PD-1, PD-L1, CSF1R, and EGFR in CF sputum neutrophils, consistent with an immunomodulatory profile.

Conclusion: HDAC11 and EZH2 inhibitors normalize key GRIM neutrophil features—bacterial killing, surface phenotype, and EV composition—without major changes in gene expression, highlighting post-translational regulation. These findings support targeting chromatin-modifying enzymes to rapidly restore neutrophil function and reduce inflammation in CF lung disease.

Hidden in Plain Sight: Rediscovering Mast Cells in Cystic Fibrosis

Authors: Miralda, Irina; Moran, John J.; Shrestha, Mahesh K.; Ozuna, Hazel; Durfey, Samantha L.; and Kopp, Benjamin T.

Presenting Author: Irina Miralda Molina, PhD

Poster Number: 24

Background: Despite partial restoration of Cystic Fibrosis Transmembrane Conductance Receptor (CFTR) function by modulator therapy, persistent immune dysfunction and bacterial infections remain significant challenges in managing Cystic Fibrosis disease. Without a comprehensive understanding of the contributors to chronic inflammation, it will be challenging to develop targeted therapies for immune dysfunction in people with CF (pwCF). Mast cells (MC) are tissue-resident immune cells with critical roles in lung homeostasis and disease. Despite their low abundance, dysregulated MC activation in the airways is linked to asthma, COPD, and fibrotic lung diseases. Nonetheless, the role of MC in CF pathology is not well defined.

Methods: Peripheral blood CD34+ progenitors from healthy controls (HC) and pwCF were differentiated into MC in vitro. HC MC and CF MC were cultured with elexacaftor/tezacaftor/ivacaftor (ETI), and we determined CFTR expression and function, assessed changes in MC markers and phenotypes, and quantified changes in activation. MC intracellular calcium influx, degranulation, and cytokine production were used as readouts for MC activation. Transcriptional changes in MC were analyzed in published single-cell RNA sequencing data from nasal brushings of healthy controls and pwCF before and after ETI treatment (Loske et al, AJRCCM, 2024).

Results: Primary human HC MC express CFTR, whose expression can be partially restored in CF MC with ETI treatment. Flow cytometry analysis showed that CF MC express more c-kit (Stem Cell Factor receptor) but fewer FCεRIα (High affinity IgE receptor) than HC MC, which have strong functional implications. CF MC have elevated intracellular calcium at baseline, but upon activation, the calcium influx is less robust compared to HC MC. Additionally, CF MC could not control bacterial growth and reduced macrophage phagocytosis and bacterial killing. Finally, while ETI drives monocytes and neutrophils toward a transcriptional state closer to homeostasis, ETI induced a distinct transcriptional signature in MC that remains divergent from healthy controls.

Conclusions: MC are dysfunctional in CF and play an underappreciated role in inflammation and innate defenses in CF. MC represent a new immunotherapeutic target in CF.

Aberrant Catecholamine Utilization by Macrophages in Cystic Fibrosis

Authors: Ozuna, Hazel; Lyles, James T.; Chandler, Joshua D.; Murphy, Ashley T.; Miralda, Irina; Durfey, Samantha L.; Moran, John J.; Shrestha, Mahesh K.; and Kopp, Benjamin T.

Presenting Author: Hazel Ozuna, PhD

Poster Number: 25

Background: Cystic fibrosis (CF) macrophages have impaired autophagy and reactive oxygen species production, reducing bacterial killing. While elexacaftor/tezacaftor/ivacaftor (ETI) improves outcomes, persistent infection and inflammation suggest additional immune dysfunction in people with CF (pwCF). We previously showed that AR-13, a COX-2 inhibitor, enhances autophagy and lowers Pseudomonas aeruginosa and MRSA burden when combined with antibiotics. Catecholamines (CAT), such as epinephrine, regulate macrophage activation, cytokine secretion, and phagocytosis. Though CAT levels are elevated in pwCF, their immune role is unclear. We hypothesize that dysregulated immuno-hormonal signaling impairs macrophage function, potentially reversible by ETI+AR-13.

Methods: Peripheral blood monocytes from pwCF and healthy controls (HC) were differentiated into monocyte-derived macrophages (MDM). To assess cytokine production, MDM were treated with ETI ± AR-13, then challenged with Pa (MOI 2). Supernatants were collected before and after infection and analyzed using the LEGENDplex Human Inflammation panel. CAT release and storage were assessed after 30min to 24hr stimulation with concanavalin A (ConA), lipopolysaccharide (LPS), phytohemagglutinin (PHA), IFNβ, PHA+IFNβ, heat-killed Pa, C5a, or IL-4. Epinephrine levels in culture supernatants and lysates were quantified via ELISA.

Results: At baseline, CF MDM expressed lower MCP-1, IL-8, and IL-18 compared to HC. Post-infection, CF MDM secreted significantly less IL-1β, TNF-α, IL-6, IL-10, MCP-1, IL-8, and IL-18, with IL-1β most reduced. Cytokine responses were not substantially rescued by ETI or AR-13. CF MDM also released less epi and retained more intracellularly, indicating a secretion defect. Release kinetics varied by stimulus: PHA and C5a (30 min), LPS (2 hr), ConA (8 hr).

Conclusion: CF MDM demonstrated impaired cytokine secretion and decreased release and increased intracellular retention of epi suggesting altered immuno-hormonal signaling in CF. Ongoing studies will focus on the impact of exogenous CAT in cytokine secretion in CF, targeted metabolomics will aim to detect additional CAT and biosynthetic enzymes, while spatial omics and CyTOF will examine adrenergic and activation receptor signaling involved in hormonal signaling defects. This work will reveal how disrupted catecholamine pathways impair macrophage function and guide new ways to strengthen host defense in pwCF.

NeoCLIP: A Self-Supervised Foundation Model for the Interpretation of Neonatal Radiographs

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Presenting Author: Puneet Sharma, MD

Poster Number: 57

Background: Artificial intelligence (AI) based on deep learning has shown promise in adult and pediatric populations in the interpretation of medical imaging to make important diagnostic and management recommendations. However, there has been little work developing new AI methods for neonatal populations. The objective of our study was to develop a novel, deep contrastive learning model to predict a comprehensive set of pathologies from radiographs relevant to neonatal intensive care.

Methods: We identified a retrospective cohort of infants who obtained a radiograph while admitted to a large neonatal intensive care unit in Boston, MA from January 2008 to December 2023. After collecting radiographs with corresponding reports and relevant demographics for all subjects, we randomized the cohort into three sets: training (80%), validation (10%), and test (10%). We developed a deep learning model, NeoCLIP, to identify 15 unique pathologies and 5 medical devices relevant to neonatal intensive care on plain film radiographs. The pathologies were automatically extracted from radiology reports using a custom pipeline based on large language models. We compared the performance of our model against various baseline methods.

Results: We identified 4,629 infants which were randomized into the training (3,731 infants), validation (419 infants), and test (479 infants) sets. In total, we collected 20,154 radiographs with a corresponding 15,795 reports. The AUROC of our model was greater than all baseline methods for every radiographic finding other than portal venous gas. The addition of demographics improved the AUROC of our model for all findings, but the difference was not statistically significant.

Interpretation: NeoCLIP successfully identified a broad set of pathologies and medical devices on neonatal radiographs, outperforming similar models developed for adult populations. This represents the first such application of advanced AI methodologies to interpret neonatal radiographs.

Sex-Based Differences in Viral Outcomes of ART-treated SIV Infection

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Presenting Author: Soham Sonawane, BA

Poster Number: 49

Background: Women make up the majority of people living with HIV globally but remain underrepresented in cure research. Biological sex influences HIV pathogenesis, as women often show stronger immune responses and lower viral set points during acute infection. However, studies on viral persistence during antiretroviral therapy (ART) have produced inconsistent results, highlighting the need for more research into how sex impacts HIV outcomes and the development of effective cures.

Methods: We conducted a meta-analysis of 18 studies to explore the impact of biological sex on viral persistence in the rhesus macaque (RM) SIV/SHIV infection model. Inclusion criteria included infant or adult studies of SIV or SHIV infection and daily ART treatment with data on viral dynamics and reservoir size. Data were extracted from 296 RMs, 70% (207) male, and 86% (142) adults. Variables included viral strain, plasma viral load (PVL), ART duration, and reservoir measures in peripheral blood (PB), lymph node (LN), and gastrointestinal (GI) tract. We compared peak PVL, cellassociated viral DNA, and intact proviral DNA levels by sex.

Results: Female adult macaques had significantly higher peak PVL compared to males (median 1.8×107 versus 1.3×107 copies/ml plasma, p=0.0062). Among SIV-infected RMs treated with suppressive ART, females had significantly higher SIV DNA levels in CD4+ T-cells from PB (2.1×103 versus 3.4×102 copies/million cells, p<0.001), LN (median 2.1×103 versus 4.4×102 copies/million cells p=0.0164), and GI tract (median 3.3×101 versus 7.7 copies/million cells, p=0.0004). Levels of CD4+ T-cells with intact provirus were similar in females and males (PB: 1.2×103 versus 8.8×102 copies/million cells, p=0.1604; LN: 1.2×103 versus 9.2×102 copies/million cells, p=0.7074). No significant differences were observed in SHIV-infected RMs.

Conclusion: These analyses revealed that, compared to males, females have higher viral loads prior to ART, a greater number of infected cells in multiple tissues during ART, but similar intact viral reservoir size. Underlying drivers may include enhanced viral spread pre-ART due to increased immune activation combined with clearance of intact reservoirs during ART due to better antiviral immunity in females. Our data support increased inclusion of women in HIV cure research for more accurate assessment of the impact of cure-directed interventions on virus persistence.

Mapping the HPV16 E6 Affinity Interactome

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Poster Number: 50

Protein interaction networks encompass the entirety of a cell's protein-protein interactions creating dynamic networks that are temporal, often transient, and contextual by nature. These networks, or interactomes, determine cellular function and regulation thus underpinning the development of disease. This places interactomes at a critical crossroads, linking genotype and phenotype and holding key insights into pathogenesis. Human Papilloma Viruses (HPVs) have often been subject to interactomic studies as they are highly diverse genotypically and phenotypically, resulting in clinical presentations that range from transient and asymptomatic to persistent and cancer causing. HPVs, with >200 isotypes, repeatedly infect people of all ages in the skin, genitals, and throat. While prophylactic vaccination against high risk-HPV (hr-HPV) types has made strides in reducing morbidities from HPV-related pathologies, most causal infections are acquired during adolescence. This results in nearly 690,00 annual cases of HPV-induced cancers, with low-and-middle income countries disproportionately affected. By conducting a high throughput quantitative interatomic study of the HPV16 virally encoded oncoprotein, E6, we've gained new insights into pathways involved in HPV driven carcinogenesis and identified potential therapeutic strategies for targeting hr-HPV infections. Here, we've applied a novel approach, known as the native holdup, to measure approximately 4500 affinities between 16E6 and endogenous host proteins present in HaCaT (HPV -) and HeLa (HPV+) cell lines. With this, a 16E6 affinity profile was generated, identifying 100 partners of interest including both previously characterized 16E6 partners, UBE3A, and TP53, and novel partners such as PTPN14, an established target of the other major oncoprotein E7. By combining this affinity profile with the quantified proteome, we were able to model the probability of these interactions occurring at different stages of infection and identify critical pathways involved in carcinogenesis. Further investigation of the 16E6-UBE3A interaction revealed the involvement of UBE3A oligomerization which represents a unique biophysical feature of HPV infections that can be exploited to develop highly specific host-directed therapeutics. In taking a quantitative approach to interactomics, we were able to characterize the 16E6 interactome at a depth and complexity never seen before.

Experimental CD8 Cell Depletion in ART-Suppressed SIV-Infected Rhesus Macaque Infants to Define the Role of CD8+ T Cells in Pediatric HIV Reservoir Maintenance

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Presenting Author: Shahab Zaki Pour, PhD

Poster Number: 51

Background: Despite antiretroviral therapy (ART) reducing morbidity and mortality in children living with HIV, there is no cure for HIV that persists in a latent reservoir. Mounting evidence implicates CD8+ T cells in controlling HIV persistence in adults. However, the influence of CD8+ T cell antiviral activities on HIV reservoir in infants is largely unknown. Given the distinct features of the developing immune system, pediatric studies are warranted. Here we conducted a proof-of-principle in vivo study to assess the impact of experimental CD8+ T cell depletion on the viral reservoir in a model of ART-treated perinatally SIV-infected rhesus macaques (RM) infants.

Methods: Sixteen RMs were infected i.v. with 5,000 IU of barcoded SIVmac239M and initiated on ART 4 weeks post-infection (wpi). After >3 months of plasma viral load (PVL) suppression on ART, animals are divided in 2 groups: 6 RMs are maintained on ART only and serve as controls while 10 RMs receive the experimental treatment consisting of a dose of the anti-CD8α-depleting antibody MT807R1 at 50 mg/kg alone or in combination with weekly doses of the latency reversing agent AZD5582 at 0.2 mg/kg for 5 weeks. A comprehensive assessment of serum chemistries, complete blood counts, body weight, PVL, and flow cytometric immunophenotyping are performed throughout the study.

Results: All infant RMs were successfully infected after one SIVmac239M challenge with a peak PVL of 1.3.107-2.5.108 SIV RNA copies per ml of plasma at 1-4 wpi. ART initiation was followed by a drastic reduction in PVL, reaching a first undetectable value in most animals by 12 wpi. The experimental treatment was initiated in 6 RM infants including 3 RMs that received the CD8 depleting antibody alone and 3 RMs that received MT807R1 and AZD5582. No adverse events were observed in these animals. A depletion of >99% of peripheral CD8+ T cells was observed 1-day after MT807R1 administration.

Conclusions: In this study, administration of an anti-CD8α-depleting antibody, alone or with the latency reversal agent AZD5582 in ART-suppressed SIV-infected RMs was well-tolerated and resulted in a significant CD8+ T cell depletion, providing a model to investigate the role of CD8+ T cells in pediatric SIV/HIV persistence.

Per- and Polyfluoroalkyl Substances (PFASs) Induce Cardiotoxicity and Alter ECM, Metabolism, and Mitochondrial Protein Profiles in Human Induced Pluripotent Stem Cell-derived Cardiomyocytes

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Presenting Author: Wenhao Zhang, PhD

Poster Number: 19

Background: Per- and polyfluoroalkyl substances (PFAS) are synthetic environmental contaminants commonly used in consumer and industrial products. Growing evidence links PFAS exposure to cardiotoxic effects, particularly during fetal development. However, the cardiotoxic potential of combined PFAS exposure, which more closely reflects real-world environmental conditions, remains poorly understood. This study aims to investigate the cytotoxic and molecular effects of combined PFAS exposure using an in vitro cardiac toxicity model developed with human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs).

Methods: hiPSC-CMs were exposed to a mixture of three common PFAS compounds—perfluorohexanesulfonic acid (PFHxS), perfluorooctanoic acid (PFOA), and perfluorodecanoic acid (PFDA) or individual PFAS compounds. Cytotoxicity, mitochondrial features, and redox balance were evaluated using a cell viability assay, TMRM, MitoTracker Red, and High-performance liquid chromatography (HPLC) analyses. Immunocytochemistry was employed to examine fibroblast and cardiomyocyte markers. Additionally, proteomic profiling was performed to identify protein levels and pathways disrupted by PFAS exposure.

Result: Compared with treatments with single PFAS compounds, combined PFAS exposure induced synergistic cytotoxicity, significantly reducing hiPSC-CM viability after 5 or 10 days of exposure. We then focused on further analyses using combined PFASs. Combined PFAS exposure for 10 days substantially reduced mitochondrial membrane potential and content in a dose-dependent manner. Redox analysis showed a shift in cysteine metabolism, potentially indicating an adaptive response to oxidative stress after 10 days of exposure. After 14 days of exposure, combined PFASs enhanced vimentin expression in cultures as detected by immunostaining, indicating fibrotic remodeling. Proteomic profiling identified that combined PFASs upregulated proteins related to extracellular matrix organization, cholesterol metabolism, and antioxidant defense pathways, and downregulated proteins related to mitochondrial function and ribosomal subunit proteins.

Conclusion: These findings demonstrate that exposure to combined PFASs induces pronounced cytotoxic and mitochondrial damages in hiPSC-CMs, with molecular signatures of mitochondrial dysfunction, fibrosis, metabolic dysregulation, and a shift in cysteine metabolism potentially indicative of an adaptive response to oxidative challenges. The results underscore the importance of evaluating PFAS mixtures to better understand cardiac risks associated with environmental exposure.

**Deep Neutrophil Phenotyping Unveils the Impact of Complement Activation in Sickle Cell Disease

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Presenting Author: William Briones, BS

Poster Number: 30

Background: Sickle cell disease (SCD) impacts millions globally. Acute chest syndrome (ACS) is a leading cause of admission to ICU and death among SCD patients. Our clinical preliminary data show that complement activation is elevated during ACS. Corroborating this, a murine model of SCD found that complement activation plays a role in driving ACS and associates with lung neutrophil extracellular trap (NET) formation (Chonat et al., STM 2025). Given these data, we hypothesized that hemolysis-mediated complement activation primes neutrophils to generate NETs, resulting in endothelial damage and ACS.

Methods: Blood collected from SCD patients experiencing ACS (n=8) and at baseline (n=7) was used to phenotype neutrophils and measure complement deposition on neutrophils using high dimensional flow cytometry. In addition, novel sickle cell (SS) mice deficient in complement component 3 (C3) (SSC3KO) were treated with hemin, and tested for complement activation and NET formation.

Results: Consistent with the established role of complement in neutrophil activation in SCD mice, Unifold Multiparameter Approximation and Projection (UMAP) analysis demonstrated increased C3 deposition on neutrophils from ACS patients compared to those from patients at baseline (3% vs. 0.6%, respectively; $p<0.01$). ACS patients were also found to have increased immature neutrophils compared to those at baseline (3% vs. 0.2%, respectively; $p<0.001$), and that C3 deposition occurred on ~16% of immature ACS neutrophils. Moreover, of the immature ACS neutrophils, 58% had a phenotype of reverse transendothelial migration (neutrophils that had entered tissue but migrated back into circulation), with 26% of these cells having C3 deposition. While C3 deposition on mature neutrophils (3.4% ACS vs 0.8% baseline; $p<0.05$) did not associate with altered complement regulatory protein expression, the majority of immature ACS neutrophils had decreased complement regulatory protein expression. Consistent with this, hemin-treated SSC3KO mice failed to develop ACS, and lacked NET formation and P-selectin expression in the lungs compared to hemin-treated wild type mice.

Conclusion: These new findings suggest that neutrophil activation during ACS is modulated by systemic complement activation. Ongoing studies in our group are evaluating how complement recruits and primes neutrophils for NET formation and endothelial dysfunction using biochemical and genomic tools.

**Rapid-Fire Finalist

**Safety Evaluation of Temporal Interference (TI) Stimulation in Mouse Hippocampus

Authors: Hou, Richard; Acerbo, Emma; Berglund, Ken; Laxpati, Neal Gordon; and Gutekunst, Claire-Anne

Presenting Author: Richard Hou

Poster Number: 60

Temporal Interference (TI) stimulation is a promising alternative to invasive deep brain stimulation (DBS) for treating neurological disorders such as pediatric epilepsy. TI uses two high-frequency electric fields generated from electrodes on the scalp with slight frequency offsets to create low-frequency amplitude-modulated waveforms. It can target deep brain regions without significantly affecting healthy tissues. This treatment benefits pediatric patients who are unable to get a neuromodulatory device due to age or size. However, concerns about potential tissue damage during high-amplitude, low-frequency modulation remain as previous studies have shown that low-frequency modulation can produce lesions in the brain tissue. In this study, we assessed safety of TI stimulation using in vitro as well as in vivo models.

We first used an egg white model, leveraging its protein similarity to those in the brain. We assessed the coagulation risks from the low-frequency stimulation. 20 minutes of TI stimulation (1 kHz and 1.005 kHz, 10 mA for 5 Hz amplitude-modulated signal at the targeted region) did not produce any obvious coagulation. As a positive control, 20 minutes of transcranial alternating current stimulation (5Hz, 10mA,) did induce localized coagulation.

We further validated safety of TI stimulation in mice. We targeted TI stimulation (1 kHz and 1.005 kHz, 2 mA, 20 minutes) to the hippocampus unilaterally. In one cohort of mice, temperature at the target site was recorded during stimulation using a thermistor. A mild increase (~0.7°C) was observed in the brain. A histological analysis was conducted in another cohort of mice. Similarly stimulated brains were collected and stained for markers of gliosis (GFAP), heat stress response (HSP70), and vasodilation (iNOS), and compared with groups with no stimulation and sham stimulation (1 kHz and 1kHz, 2 mA, 20 minutes). Notably, GFAP expression was increased in the stratum lacunosum moleculare, suggesting possible localized brain injury. On the contrary, the expression of HSP70 and iNOS was minimal with no significant increase in the hippocampus, indicating that inflammation and vasodilation were limited.

These findings suggest that gross thermal effects were relatively small during TI stimulation and that it does not trigger significant injury or widespread inflammatory responses.

** Rapid-Fire Finalist

**Therapeutic Intervention with Meclizine Ameliorates Mitochondrial Ultrastructural Defects in the Phosphate Carrier Deletion Model of Primary Mitochondrial Cardiomyopathy

Authors: Huang, Benjamin; Ghazal, Nasab; and Kwong, Jennifer

Presenting Author: Benjamin Huang

Poster Number: 16

Background: Mutations in mitochondrial proteins can alter their structure and function, disrupting cellular energy production and contributing to primary mitochondrial disorders. These disorders often affect tissues with high energy demands, such as the heart. Mitochondria-related heart failure accounts for 20%-40% of cases in children with mitochondrial disorders and remains incurable.

Our lab has previously developed a model of inducible cardiac mitochondrial energy dysfunction with the cardiomyocyte specific deletion of the mitochondrial phosphate carrier (SLC25A3, cKO mice). SLC25A3 encodes a mitochondrial transporter that imports inorganic phosphate into the mitochondrial matrix for ATP synthesis, and thus, is a critical component of the mitochondrial energy production machinery. Following onset of SLC25A3 deletion, cKO mice develop mitochondrial cardiomyopathy characterized by impaired mitochondrial ATP synthesis, mitochondrial hyperproliferation and ultrastructural disorganization, myofibril misalignment, and impaired heart function. Critically, despite reduced mitochondrial ATP synthesis, the total cardiac ATP levels are maintained, suggesting a protective upregulation energy production through glycolysis.

We hypothesize that strategies to upregulate glycolysis are protective in the context of primary mitochondrial energy dysfunction. In this study, we use meclizine, an FDA approved drug that was found to shift energy metabolism to glycolysis, to test its effect on mitochondrial energy dysfunction in the heart.

Methods: SLC25A3 deletion was induced with tamoxifen in 2-month-old SLC25A3 cKO mice and subsequently administered vehicle or meclizine by oral gavage for 12 weeks. Following treatment, hearts were collected and fixed in glutaraldehyde, post-fixed with osmium tetroxide, and embedded in resin for electron microscopy to examine mitochondrial ultrastructure and myofibril organization. ImageJ was used to measure mitochondria size and shape.

Results: Electron microscopy revealed that meclizine significantly ameliorated the myofibril disarray in SLC25A3 deleted hearts. Additionally, while SLC25A3 cKO hearts displayed disrupted mitochondrial ultrastructure and extensive mitochondrial hyperproliferation, these metrics were restored with meclizine treatment, proven by significant increase in mitochondrial area, perimeter and Feret's diameter.

Conclusions: Meclizine treatment reduces mitochondrial hyperproliferation and restore myofibril organization in the SLC25A3 cKO model of mitochondrial energy dysfunction and primary mitochondrial cardiomyopathy. Future identification of the mechanisms by which meclizine confers these protective effects may provide directions for therapy developing for primary mitochondrial disease.

** Rapid-Fire Finalist

**Biomarkers of Endothelial Activation in Pediatric Sepsis and an *In Vitro* Sepsis Model

Authors: Ripple, Michael; Fitzpatrick, Anne; Kopp, Benjamin; and Grunwell, Jocelyn

Presenting Author: Michael Ripple, MD, PhD

Poster Number: 46

Background: Sepsis is a life-threatening condition marked by immune dysregulation and systemic inflammation in response to infection, often leading to organ dysfunction. Endothelial cells are activated early in the inflammatory cascade and orchestrate leukocyte recruitment, cytokine release, and microvascular injury. Circulating markers of endothelial activation have been associated with multi-organ failure and a high mortality phenotype in pediatric sepsis. However, we lack targeted therapies for endothelial activation in sepsis due to an incomplete understanding of underlying mechanisms.

Objective: To determine whether biomarkers of endothelial activation are elevated in pediatric patients with sepsis compared to patients with non-sepsis critical illness and in an in vitro model of sepsis.

Methods: We measured levels of four biomarkers of endothelial activation (intercellular adhesion molecule-1 (ICAM-1), angiopoietin-2 (Ang-2), IL-8, and soluble thrombomodulin (sTM)) in plasma from pediatric patients with sepsis and non-sepsis critical illness. We also measured levels of ICAM-1, Ang-2, IL-8, and sTM in supernatant from human umbilical vein endothelial cells (HUVECs) treated with a sepsis cytokine mixture and LPS in an in vitro sepsis model. Biomarker levels were measured using sandwich ELISA.

Results: We found a significant increase in levels of ICAM-1, Ang-2, and IL-8 in pediatric patients with sepsis compared to patients with non-sepsis critical illness. HUVECs treated with a sepsis cytokine mixture or LPS had higher levels of ICAM-1, IL-8, and sTM in the cell culture supernatant compared to untreated cells. Transcription and expression of biomarkers were confirmed with qPCR and immunofluorescence.

Conclusions: Biomarkers of endothelial activation are associated with poor outcomes in pediatric sepsis. ICAM-1, Ang-2, IL-8, and sTM are elevated in pediatric patients with sepsis and in an in vitro sepsis model. These findings support the role of endothelial activation in the pathobiology of pediatric sepsis and highlight these biomarkers as potential targets for therapeutic strategies in the endothelium. The concordance between clinical and in vitro results strengthens the validity of this model for further mechanistic studies. Future work will aim to define the pathways driving biomarker release and their contribution to organ dysfunction.

** Rapid-Fire Finalist

**Developing a Dual-Targeting CAR T-cell Strategy Against Pediatric Mixed Phenotype Acute Leukemia (MPAL)

Authors: Sullivan, Emily C.; Patel, Vishva; La Fuente, Moira; Branella, Gianna M.; and Raikar, Sunil S.

Presenting Author: Emily Sullivan, BS

Poster Number: 9

Background: Mixed phenotype acute leukemia (MPAL) accounts for 2-5% of acute leukemias and is characterized by the presence of both lymphoid and myeloid phenotypes. Expression of lymphoid and myeloid lineage markers can either be in a biphenotypic pattern (expressed on the same cell) or a bilineal pattern (multiple lineage specific blast populations), or a combination of both. Furthermore, MPAL blasts have been reported to undergo lineage plasticity, switching between lymphoid and myeloid immunophenotypes. This heterogeneity of MPAL has led to a lack of standard treatment, and there remains an unmet need for MPAL-specific therapeutics. We aimed to develop a dual antigen-targeting chimeric antigen receptor (CAR) T cell approach, targeting both the B-lymphoid antigen CD19 and the myeloid antigen CD33, to effectively target B/myeloid MPAL.

Methods: A mixed biphenotypic/bilineal MPAL was recapitulated in the form of the CD19/CD33-expressing B/myeloid MPAL JIH-5 cell line, while a uniquely bilineal model was created by coculturing the B-ALL RS4-11 and AML MV4-11 cell lines. Pediatric primary patient samples were assessed for CD19/CD33 surface expression. Lineage plasticity was examined via flow cytometry using our JIH-5 cell line. We created a pooled dual-targeting CAR T cell approach, targeting CD19 and CD33, and assessed its cytotoxicity against our models of biphenotypic and bilineal MPAL.

Results: Flow cytometry analysis of pediatric B/myeloid MPAL primary patient samples confirmed the heterogeneous expression pattern of CD19 and CD33 in blast cells necessitating a dual-antigen approach. JIH-5 MPAL blasts that were single-positive for CD19 were flow sorted from their double-positive CD19/CD33 counterparts became double-positive over time, confirming lineage plasticity. Our pooled CAR approach exhibited superior cytotoxicity to either single CAR approach against both the JIH-5 model as well as our bilineal model; however, both single CAR approaches exhibited impressive antigen-nonspecific killing of tumor cells, termed “bystander effect”.

Conclusions: Our studies show that a dual lymphoid/myeloid antigen targeting CAR approach is needed to overcome blast heterogeneity in B/myeloid MPAL. The optimal dual CD19/CD33 CAR strategy is currently being investigated, and we plan to validate our approach in both cell line and patient derived xenograft models of B/myeloid MPAL.

** Rapid-Fire Finalist

**Investigating the Role of SIX1 and EYA2 in T-ALL Leukemogenesis using CRISPR-Cas9-Mediated Knockout in Jurkat Leukemia Cells

Authors: Varma, Suhani; Shen, Huifeng; Alexander, Lyndsey; Wechsler, Daniel; and Aumann, Waitman

Presenting Author: Suhani Varma

Poster Number: 11

Background: The CALM-AF10 fusion is a recurrent chromosomal translocation associated with highly aggressive leukemias, including T-cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML). CALM-AF10 leukemia is consistently linked to poor clinical outcomes, highlighting the need for novel therapeutic interventions. We previously identified SIX1 as a downstream effector gene of CALM-AF10, with elevated SIX1 expression associated with worse survival in both T-ALL and AML. As SIX1 relies on its transcriptional cofactor EYA2, this study investigates the roles of SIX1 and EYA2 using Jurkat cells as a model.

Methods: Single-guide RNAs (sgRNAs) against SIX1 and EYA2 and a non-targeting scramble control were inserted into lentiviral vectors containing a blue fluorescent protein (BFP) reporter. These plasmids were amplified in Stbl3 E. coli and electroporated into Jurkat cells stably expressing Cas9 (Jurkat-Cas9). Following transfection, cells that picked up the sgRNAs were isolated by fluorescence-activated cell sorting (FACS) on BFP, expanded in culture, and assessed for gene disruption using immunoblotting, PCR, and Sanger sequencing. Cell proliferation was tracked over a 72-hour period.

Results: 75% of Jurkat-Cas9 cells exhibited BFP fluorescence following FACS, indicating efficient transfection of successfully cloned sgRNAs., Western blot analysis revealed reduced SIX1 protein expression in two independent lines, with Sanger sequencing confirming successful gene knockout. Notably, cells with diminished SIX1 expression demonstrated reduced proliferation relative to scramble populations. In contrast, attempts to disrupt EYA2 were unsuccessful.

Conclusion: Reduced proliferation of Jurkat-Cas9 cells with decreased SIX1 establishes a potentially important role of SIX1 in cell survival, while the inability to knockout EYA2 knockout suggests a potentially critical role in cell survival. Defining the contributions of SIX1 and EYA2 in leukemia cells may reveal novel therapeutic vulnerabilities. Ultimately, targeting these factors could offer a promising strategy for precision medicine approaches aimed at improving clinical outcomes in patients with CALM-AF10-positive leukemia.

** Rapid-Fire Finalist

**A Tale of Two Mutations: How PHF14 Loss and Gain Variants Disrupt Brain Development

Authors: Wu, Yongji; Dominguez, Gaea; Seyoum, Eskender; Lin, Weilan; and Zhou, Jian

Presenting Author: Yongji Wu, PhD

Poster Number: 64

Background: Plant Homeodomain Finger Protein 14 (PHF14) is an epigenetic regulator with critical roles in cell proliferation and differentiation, cancer, fibrosis, and neurodevelopmental disorders (NDDs). As a chromatin-associated protein, PHF14 modulates gene expression by interacting with TCF20 and MeCP2—the causal genes for Rett Syndrome (RTT) and TCF20 associated NDD, respectively—within the TCF20/PHF14 complex. We previously identified a PHF14 missense variant (c.964T>G, p.C322G) in an NDD patient presented with clumsy gait, developmental delay/intellectual disability, speech delay, and an RTT-like regression in gross motor skills and balance. This mutation disrupts the interaction between PHF14, TCF20, and MeCP2 within the complex. Our clinical and biochemical findings highlight the strong link between TCF20/PHF14 complex and NDD pathogenesis; however, the underlying neuronal and molecular mechanisms remain unclear.

Methods: To understand the role of PHF14 in brain development, we generated Phf14 knockout (KO) and Phf14-C322G knock-in mouse models, collected the brain tissue at various developmental stages to assess the impact of PHF14 mutations on neurodevelopment by employing Western blot, immunostaining, and RNA-seq analyses.

Results: At the early stage, Phf14 deletion impairs cortical development by reducing SOX2 expression and decreasing the number of PHH3+ and Satb1/2+ cells at E17.5, suggesting that PHF14 plays an important role in regulating the mitotic activity, proliferation, and differentiation of neural progenitor cells. RNA-seq analysis reveals disrupted neurodevelopmental pathways, including neuropeptide signaling, cell-cell signaling, and neurogenesis in Phf14 KO mice. Furthermore, Phf14 KO mice exhibit neonatal lethality at P0, highlighting its essential role in development.

Notably, homozygous Phf14C322G/C322G mice exhibit an earlier embryonic lethality at E15.5, whereas heterozygous Phf14-/C322G mice remained viable into adulthood, suggesting that the C322G mutation is a potential gain-of-function variant by enhancing ectopic PHF14 functions rather than causing a complete loss of function.

Conclusion: Our study established PHF14 as a key regulator in brain development and reveals novel molecular mechanisms underlying both loss- and gain-of-function effects of PHF14 mutations, shedding light on its role in NDD pathogenesis.

** Rapid-Fire Finalist

**Impact of a Novel Mobilizer EMU116 and an Anti-sickling Agent Osivelotor on Stem Cell Mobilization in the Sickle Mouse

Authors: Yoo, Justin; Patel, Ashwin; Goldsborough, Kennedy; Hernandez, Britney; Aliche, Mary; Titus, Jedidah; Wilson, Larry; Liotta, Dennis; and Sheehan, Vivien

Presenting Author: Justin Yoo, MD

Poster Number: 36

Background: Ex vivo gene therapy (GT) can transform the lives of people with sickle cell disease (SCD). However, hematopoietic stem cell (HSC) collection is challenging because the disordered bone marrow (BM) stroma and vasculature impair sufficient HSC mobilization with plerixafor, and addition of standard mobilizing agent granulocyte colony-stimulating factor cannot be safely used due to risk of inducing sickle-related complications. In a sickle mouse model, we showed that an anti-sickling agent osivelotor can reverse the BM abnormalities in SCD. We now ask if osivelotor can improve plerixafor mobilization in the sickle mouse and separately test the efficacy of a novel mobilizer, EMU116, a CXCR4 inhibitor.

Methods: Townes sickle mice (HbSS) were fed for 8 weeks of control or osivelotor chow and mobilized with plerixafor, EMU116, or PBS subcutaneously. Peripheral blood (PB) was collected for a complete blood count (CBC), flow cytometry, and colony forming unit (CFU) assay. Non-sickle HbAA mice were also treated with plerixafor or EMU116. Median values were compared across groups using non-parametric tests.

Results: Plerixafor and EMU116 increased WBC in HbSS and HbAA animals. The percent lineage negative Sca-1cKit+ (LSK) cells as a percentage of all Lin- (%LSKLin-) cells in the PB were higher with EMU116 than PBS control in HbAA and HbSS mice. Compared to plerixafor, EMU116 mobilized a higher %LSK in HbAA (0.03% vs 0.004%, p=0.02) and HbSS mice (0.08% vs 0.005%, p=0.01). Further, EMU116 SC mobilized more Flt3-CD34- cells in HbSS compared to plerixafor (0.3% vs 0.05%, p=0.006) but comparable to HbSS+plerixafor when pretreated with osivelotor. There were higher CFUs in all mobilized groups.

Conclusions: The novel mobilizer EMU116 increases stem cells able to rapidly reconstitute (LSKLin-) and primitive HSCs with long term potential (%Flt3-CD34-) in the PB of HbSS and HbAA mice significantly more than plerixafor. The mobilized HSCs are functional, able to proliferate and differentiate as suggested by the higher number of CFUs. We conclude that EMU116 is a promising mobilizer for healthy individuals and individuals with SCD, and SCD modification with osivelotor may be an effective pretreatment strategy to improve mobilization efficiency of primitive HSCs in individuals seeking GT.

** Rapid-Fire Finalist

**Adaptive Protein Synthesis in Genetic Models of Copper Deficiency and Childhood Neurodegeneration

Authors: Lane, Alicia R; Scher, Noah E; Bhattacharjee, Shatabdi; Zlatic, Stephanie A; Roberts, Anne M; Gokhale, Avanti; Singleton, Kaela S; Duong, Duc M; McKenna, Mike; Tran, Tommy; Petris, Michael J; Cox, Daniel N.; Roberts, Blaine R; Werner, Erica; and Faundez, Victor

Presenting Author: Alicia Lane, PhD

Poster Number: 62

BACKGROUND: Rare inherited diseases caused by mutations of the copper transporters SLC31A1 (CTR1) or ATP7A induce copper deficiency in the brain and throughout the body, causing seizures and neurodegeneration in infancy. Exactly how copper dysregulation influences neuropathology in such diseases remains unclear.

OBJECTIVE: To characterize the molecular mechanisms by which neuronal cells respond to copper deficiency.

DESIGN/METHODS: We generated CTR1 KO neuroblastoma clonal cell lines, which we characterized by TMT mass spectrometry and Nanostring to report the proteome and metabolic transcriptome. Bioenergetic measures were quantified by Seahorse. Protein synthesis was measured by puromycin incorporation and Western blot. To corroborate these findings in the brain, we profiled the cerebellum of copper-deficient ATP7A flx/Y :: Vil Cre/+ mice using Nanostring GeoMx spatial transcriptomics. Finally, we tested whether increased mTOR pathway activity in copper-deficient neurons was adaptive or deleterious by genetic epistasis experiments in Drosophila.

RESULTS: CTR1 KO cells are depleted of copper and exhibited compromised copper-dependent Golgi and mitochondrial enzymes, inducing a metabolic shift favoring glycolysis over oxidative phosphorylation. These copper-deficient cells exhibited concomitant increased mTOR-S6K activation and reduced PERK signaling. Increased mTOR-S6K activation was shown to be a pro-survival mechanism that resulted in increased protein synthesis. This finding was recapitulated in copper-deficient Purkinje cells in mice, which upregulate protein synthesis machinery and mTOR-S6K pathway genes. Finally, we showed that neuronal copper deficiency phenotypes in Drosophila are rescued by S6K over-expression or 4-EBP (Thor) RNAi.

CONCLUSIONS: Increased mTOR-S6K pathway activation and protein synthesis is an adaptive mechanism by which neuronal cells respond to copper deficiency. This is the first evidence that 1) genetic defects that impair cellular copper homeostasis simultaneously modify two signaling pathways regulating protein synthesis and 2) in which the upregulation of protein synthesis is adaptive for cell-autonomous disease phenotypes. We propose that neuronal cell pathology occurs when resilience mechanisms engaged in response to copper deficiency are outpaced by the increasing bioenergetic demands of the cell during neurodevelopment.

** Rapid-Fire Finalist

**Clinically Distinct Metabotypes of Pediatric Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)

Authors: Huneault, Helaina E.; Tiwari, Pradeep; Jarrell, Zachery R.; Smith, Matthew Ryan; Chen, Chih-Yu; Ramirez Tovar, Ana; Sanchez-Torres, Cristian; Aguayo, Liliana; Gillespie, Scott; Bai, Shasha; Carrillo-Larco, Rodrigo M.; Jain, Ajay K.; Yates, Katherine P.; Neuschwander-Tetri, Brent A.; Schwimmer, Jeffrey B.; Xanthakos, Stavra A.; Molleston, Jean P.; Behling, Cynthia A.; Fishbein, Mark H.; Hartman, Terryl J.; Pasquel, Francisco J.; Kamaleswaran, Rishikesan; Jones, Dean P.; Welsh, Jean A.; and Vos, Miriam B.

Presenting Author: Ana Ramirez Tovar, MD (presenting for Helaina Huneault, PhD)

Poster Number: 53

Background: Phenotypic heterogeneity in children with metabolic dysfunction-associated steatotic liver disease (MASLD) remains poorly understood. This study aimed to identify metabolic phenotypes (metabotypes) in pediatric MASLD using clinical, histological, and high-resolution metabolomics data to inform precision healthcare interventions.

Methods: We analyzed clinical and untargeted metabolomics data from 514 children (5-18 yrs) with biopsy-confirmed MASLD from three NASH Clinical Research Network (CRN) studies. Unsupervised k-means clustering was applied using age, WC, SBP, uric acid, ALT, AST, TG, VLDL, LDL, and HOMA2-IR. Integrative network and pathway enrichment analyses were performed to examine metabolic alterations across metabotypes.

Results: Three metabotypes were identified: Early-mild (49.4%, younger, lowest lipids, liver enzymes, and insulin resistance), Cardiometabolic (36.8%, highest WC, lipids, uric acid, and SBP), and Inflammatory-fibrotic (13.8%, highest liver enzymes, steatohepatitis, and fibrosis). Network and pathway analyses revealed altered tryptophan metabolism in the Inflammatory-fibrotic metabotype ($p < 0.001$), including elevated kynurenine pathway metabolites, which were correlated with fibrosis ($p < 0.05$). Altered BCAA degradation, butanoate metabolism, and purine metabolism were identified in the Cardiometabolic metabotype ($p < 0.05$). The Early-mild group exhibited fewer metabolomic alterations, suggesting less severe disease.

Conclusions: This study highlights significant heterogeneity in pediatric MASLD, identifying three clinically distinct metabotypes with unique metabolomic profiles and disease severity. These novel findings provide a framework for the development of precision nutrition interventions tailored to each metabotype, aligning dietary strategies with underlying metabolic pathways and disease mechanisms. Further research is needed to validate these metabotypes and advance precision healthcare for pediatric MASLD.

**Rapid-Fire Finalist

**Impact of State-Level Food Insecurity on Overall and Cancer-Related Five-Year Survival in Children with Leukemia

Authors: Webster, Caitlin; Gary, Rebecca; Wood, Kathryn; Zhang, Wenhui; and Bai, Jinbing

Presenting Author: Caitlin Webster, MSN, RN, CPHON

Poster Number: 12

Background: Leukemia is the most prominent childhood cancer, accounting for nearly one-third of pediatric diagnoses. Significant improvements in survival have been observed due to recent medical advancements. However, Black and Hispanic children with leukemia experience disparate survival outcomes. Food insecurity, defined as hindered access to sufficient amounts of safe and nutritious foods, is one factor that may contribute to these inequitable outcomes. Minority populations and cancer communities experience an increased prevalence of food insecurity, with recent research demonstrating significant associations between food insecurity and survival in adult cancer patients. No studies have investigated these associations in pediatric oncology populations.

Purpose/Significance: This study aimed to examine the influence of food insecurity severity on overall and cancer-related five-year survival in children with leukemia.

Methods: Children (0-19 years) with leukemia from 2012-2017 were collected from the Surveillance, Epidemiology, and End Results program. State-level household food insecurity prevalence was gathered from the Food Environment Atlas and cross-referenced with the state where participants were diagnosed. Kaplan-Meier estimates and Cox proportional hazard models examined associations between food insecurity severity and overall and cancer-related five-year survival. Final models were stratified by race and ethnicity to explore underlying factors contributing to disparate survival outcomes.

Results: Participants (n=6213) were primarily 1-4 years of age (37.97%), male (56.06%), Hispanic (41.35%), and diagnosed with acute lymphocytic leukemia (75.26%). The median survival time was 45 months (IQR=33). Most participants had moderate state-level food insecurity (56.43%). Survival analyses showed that low food insecurity reduced the risk of mortality compared to high food insecurity when adjusting for sociodemographic and clinical covariates (Overall: aHR = 0.72, 95% CI: 0.58-0.90, p = 0.004; Cancer-related: aHR = 0.71, 95% CI: 0.56-0.90, p = 0.004). Low food insecurity was no longer a statistically significant protective factor against mortality when stratified by race and ethnicity.

Conclusion: Findings suggest that food insecurity may influence five-year survival in children with leukemia. However, potential factors associated with race and ethnicity might alter the significance of this relationship, such as supermarket redlining. Future research that includes additional covariates and utilizes household-level food insecurity measures is needed.

**Rapid-Fire Finalist

Determinants of Linkage to Care in a Novel Newborn Screening Program for Sickle Cell Disease in Ghana, West Africa

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Presenting Author: Tarun Aurora, MD, MSCI

Poster Number: 28

Background: Sickle cell disease (SCD) is a major public health concern in Ghana, with a birth prevalence of up to 2%. Early diagnosis through newborn screening (NBS) significantly improves outcomes by enabling prompt management, yet linkage to care remains a challenge. Studies in Western Africa reveal that only ~50% of diagnosed infants are linked to care, undermining the benefits of NBS. In 2024, a NBS program was launched at Tamale Teaching Hospital (TTH) in northern Ghana, referring diagnosed infants for specialized SCD care.

Objective: We report early outcomes of care linkage and explore barriers and facilitators to care engagement among newly diagnosed infants with SCD.

Methods: Using the HemoTypeSC™ point-of-care test kit, we screened neonates at TTH and infants up to 9 months attending the child welfare (immunization) clinic. This test detects hemoglobin variants using heel-prick blood samples. Families of infants diagnosed with Hb SS or SC were counseled, enrolled in follow-up, and reminded of upcoming appointments. Written consent was obtained for longitudinal follow-up.

Results: In the first 56 weeks, 5,252 infants were screened: 3,952 (75.24%) newborns and 1,300 (24.75%) infants. Male infants comprised 50.97% of those screened. Genotype distribution included HbAA 74.43%, HbAC 14.22%, HbAS 8.83%, HbCC 1.23%, HbSC 0.97%, and HbSS 0.30%, yielding an SCD birth prevalence of 1.28% and a trait prevalence of 23.06%. All families (100%) agreed to clinic enrollment, and all (100%) received phone call reminders, yet only 34 (50.7%) of 67 SCD patients attended their first clinic appointment.

Barriers to attending first clinic appointment included perceived health of the child, disagreement with the diagnosis, self-management by healthcare worker parents, financial constraints, religious beliefs, and long distances to the hospital. Families of patients with Hb SS, greater average distance from the facility (42.1 km vs. 35.2 km), and higher caretaker education levels were more likely to miss appointments.

Conclusion: While the NBS program identified an SCD birth prevalence of 1.3%, only half of diagnosed infants linked to care, underscoring barriers such as low disease literacy, stigma, and logistical challenges. Addressing these barriers through qualitative research and tailored interventions is critical to improving care engagement in Ghana.

Clinical Predictors of Fatality in Pediatric Rocky Mountain Spotted Fever Cases in Sonora, Mexico 2004-2024

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Presenting Author: Stephanie Bellman, PhD

Poster Number: 37

Background: Rocky Mountain Spotted Fever (RMSF) is a severe and fatal illness caused by the bacterium Rickettsia rickettsii. Over the past two decades, fatality rates in the pediatric population of Sonora, Mexico have been 3x higher than across the border in the U.S. This study evaluates the clinical and demographic features associated with RMSF in the pediatric population of Sonora.

Methods: We conducted a retrospective analysis of 500 cases of hospitalized pediatric RMSF cases in Sonora, Mexico from January 2004 to December 2024. Descriptive statistics and multivariate logistic regression were used to analyze factors associated with fatal infections.

Results: The case fatality rate (CRF) in this cohort was 19.8% across the entire time period with a CFR of 31.4% before 2014 and 14.5% from 2014-2024. The median time from symptom onset to hospitalization was 5 days. Across the entire time period, fatality was significantly associated with having a delay in treatment with doxycycline past 5 days from symptom onset. Children with fatal infection were also more likely to have higher leukocyte and neutrophil counts, lower platelets, higher liver enzymes and have vomiting, diarrhea, edema, petechia, rash on the palms and soles, neurologic symptoms, hemorrhage, and kidney injury/failure on presentation or during admission. From 2014-2024, older age was significantly associated with fatal outcomes. There was also a significant decrease in treatment delay with doxycycline over the past 10 years; providers were half as likely to delay initiating treatment after 5 days from symptom onset in 2014-2024 compared to 2004-2013.

Conclusion: RMSF is a serious public health problem in the pediatric population in Sonora, Mexico with high CFR. In the past 10 years the CFR has decreased, though more fatalities are being seen in teenagers. Increased awareness and initiation of doxycycline in children has likely driven this decrease in CFR over the past 10 years. All children presenting with fever should be evaluated for RMSF and treated immediately if suspected. Despite decrease in CFR over the past decade, RMSF remains a concern to the pediatric population and demands attention.

Maternal SSRI Exposure During Pregnancy and Neonatal Cardiopulmonary Outcomes: A Systematic Review and Meta-Analysis

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Presenting Author: Mahathi Chavali, MD

Poster Number: 56

Background: Maternal depression and anxiety are prevalent during pregnancy, often leading to the prescription of selective serotonin reuptake inhibitors (SSRIs). While essential for maternal well-being, concerns exist regarding the potential impact of in-utero SSRI exposure on neonatal cardiopulmonary outcomes, specifically the risk of congenital heart defects (CHDs).

Hypothesis: Maternal SSRI exposure is associated with an increased risk of these neonatal cardiopulmonary outcomes.

Methods: This study employed a systematic review and meta-analysis of observational studies. We comprehensively searched major electronic databases PubMed, Web of Science, and Google Scholar. Included studies compared neonatal cardiopulmonary outcomes in infants exposed to SSRIs during pregnancy with those unexposed. Primary endpoints constitute any cardiovascular malformation, and secondary endpoints include specific CHD, i.e., atrial septal defect (ASD), ventricular septal defect (VSD), and persistent pulmonary hypertension (PPHN). Pooled risk ratios (RRs) with 95% confidence intervals (CIs) were calculated using the binary random-effects model. A p-value ≤ 0.05 was considered statistically significant.

Results: A total of 22 observational (20 cohort, 2 case-control) studies with 74,850 infants born with cardiovascular malformations were included in our analysis. Pregnant women who used SSRIs had a significantly increased risk of infant cardiovascular-related malformations (RR 1.26; 1.13 - 1.39; p=0.003), ASD (RR 2.06; 1.40 - 3.03; p=0.037). Although the risk of VSD (RR 1.15; 0.97 – 1.36; p=0.197) and PPHN (RR 1.30; 0.76 – 2.21; p=0.98) were also increased in infants following maternal SSRI usage, these associations did not reach statistical significance.

Conclusion: This meta-analysis reveals a significant association between maternal SSRI exposure and an increased risk of overall cardiopulmonary adverse outcomes in infants, underscoring the need for careful risk-benefit assessment in pregnancy SSRI prescription and enhanced prenatal counseling. While non-significant trends for VSD and PPHN were observed, the findings highlight the importance of heightened postnatal surveillance for cardiac anomalies in exposed infants and call for further research into specific SSRI effects and underlying mechanisms to optimize maternal mental health care and fetal safety.

Clinical Outcomes of a Dexamethasone and Nelarabine-based Institutional Approach in T-cell Acute Lymphoblastic Leukemia

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Presenting Author: Ryan Summers, MD

Poster Number: 10

Background and Aims: Recent trials in pediatric T-cell acute lymphoblastic leukemia (T-ALL) added nelarabine to prednisone-based chemotherapy (AALL0434; disease-free survival [DFS] 88%) and substituted dexamethasone for prednisone in induction (AALL1231; event-free survival [EFS] 82%). No trial has combined dexamethasone-based induction with nelarabine-based post-induction therapy. This study describes clinical features and outcomes of children with T-ALL treated with dexamethasone-based induction and nelarabine-based post-induction (DEX+NEL).

Methods: This single-institution retrospective study included children aged 1-20 years with T-ALL diagnosed from 2019-2024. Clinical features are reported descriptively. EFS, DFS, and overall survival (OS) were calculated using the Kaplan-Meier method. Events were end-consolidation minimal residual disease positivity (MRD+, ≥0.01%), relapse/progression, or death. DFS was defined as time from diagnosis to relapse/progression, second malignancy, or death. Censoring occurred at date of last contact.

Results: The 60-patient cohort was 70% male, 56.7% White, 36.7% Black, and 18.3% Hispanic. CNS status was CNS1: 65%; CNS2: 26.7%; CNS3: 8.3%. Median presenting white blood cell count was 64.7 x 10^3/μL (range 2.2-950 x 10^3/μL). At end-induction, 54/60 (90%) patients achieved complete remission (CR); of those 24.1% were MRD+. The remaining 6 patients achieved CR by end-consolidation, with 1 remaining MRD+. End-induction MRD values were 0.01-1% (n=8), 1%-10% (n=6; 2 end-consolidation MRD+), and >10% (n=5; 1 end-consolidation MRD+). Median follow-up duration was 34.5 months (range 2-64). Three-year EFS, DFS, and OS was 89.6% (95% CI 78.4-95.2), 91.1% (95% CI 79.9-96.2), and 90.7% (95% CI 78.9-96.0%), respectively. First events were end-consolidation MRD+ (n=3), relapse/progression (n=2) and death (n=1); the latest event occurred 6 months from diagnosis. Deaths were from disease (n=3) and infection (n=2; 1 in consolidation, 1 after transplant).

Conclusions: This single-center cohort had excellent EFS, DFS, and OS for DEX+NEL-treated patients with T-ALL. Our data provide a strong rationale for utilizing this hybrid AALL0434/AALL1231 chemotherapy backbone in future phase 3 T-ALL trials.

Atovaquone Decreases Myeloid Production in Juvenile Myelomonocytic Leukemia

Authors: Harris, Carly; Yu, Wen-Mei; Zhao, Peng; Frank, David; and Qu, Cheng-Kui

Presenting Author: Carly Harris

Poster Number: 1

Background: Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive pediatric myeloproliferative neoplasm. This cancer has a 40% relapse rate post-transplant, highlighting the need for a novel therapeutic approach.

A significant percentage of JMML cases are caused by a gain-of-function mutation in PTPN11, which encodes the protein tyrosine phosphatase SHP2 and promotes hyperactive Ras-MAPK signaling. We previously found in a mouse model that knock-in Ptpn11E76K/+/VavCre mutant leukemic stem cells (LSCs) have diminished Stat3 phosphorylation, yet remain dependent on the Stat3 protein for survival, suggesting an exploitable vulnerability.

We aim to explore this potential vulnerability through a series of experiments utilizing control Ptpn11+/+/VavCre mice cells, knock-in Ptpn11E76K/+/VavCre mice LSCs, and FDA-approved Stat3 inhibitor Atovaquone.

Methods: To evaluate the effects of Stat3 inhibition, lineage-negative (Lin−) cells were isolated from bone marrow of Ptpn11+/+/VavCre and mutant Ptpn11E76K/+/VavCre knock-in mice using a Lineage Cell Depletion Kit and magnetic columns. Colony-forming unit granulocyte-macrophage (CFU-GM) assays were conducted to assess myeloid differentiation. Lin- Cells were cultured in vitro for 4–6 days in StemSpan media supplemented with SCF, TPO, and FLT3L, and varying concentrations of Atovaquone. Myeloid cell differentiation and apoptosis were analyzed using flow cytometry. Phospho-Stat3 status was analyzed using Western blot.

Results: Western blot confirmed reduced Stat3 phosphorylation in Ptpn11E76K/+/VavCre Lin- cells. Treatment with Atovaquone reduced both the size and number of colonies in CFU-GM assays, suggesting inhibited myeloid differentiation. In vitro culture of Ptpn11E76K/+/VavCre cells showed enhanced myeloid differentiation at baseline, and reduced differentiation following Atovaquone treatment compared to Ptpn11+/+/VavCre. Apoptosis assays revealed more apoptotic cells in Ptpn11+/+/VavCre cells compared to Ptpn11E76K/+/VavCre after Stat3 inhibitor exposure.

Conclusion: This data has been collected using Lin- cells as an experimental model, and the next step will focus on treatment of stem cells. This preliminary data supports the hypothesis that Ptpn11E76K/+/VavCre mutant LSCs are more sensitive to pharmacological inhibition of Stat3. This validates Stat3 as a promising therapeutic target in JMML.

Synergistic Effects of Venetoclax and AZD5582 In Vitro

Authors: Lin, Danielle; Ukhueduan, Benedict; and Chahroudi, Ann

Presenting Author: Danielle Lin

Poster Number: 4

Background: The persistence of latently HIV-infected CD4+ T cells remains a major barrier to a cure. Exploiting inhibitors of cell survival pathways, such as BCL-2, to eliminate infected cells is a promising solution. This study investigated the BCL-2 inhibitor Venetoclax (VTX) in combination with AZD5582, an inhibitor of the cellular inhibitor of apoptosis proteins (cIAPi). AZD5582 is known to reactivate latently HIV-infected CD4+ T cells and induce apoptosis. We hypothesized that this combination would enhance apoptosis of infected cells thereby reducing the latent HIV reservoir.

Methods: The cryopreserved ACH-2 cell line, a latently HIV-1-infected T-cell clone derived from CEM cells, was the model for HIV persistence as the virus is present but not actively replicating. Before testing the experimental drugs, Propidium Iodine (PI) and Annexin V were titrated for optimal staining. Next, five million ACH-2 cells per condition were seeded in 2 mL R10 in 6-well plates. Each treatment was performed in triplicate. Cells were stimulated with AZD5582 (100 nM) for 1 hour, washed, and then incubated with VTX (1 μM) for 24 hours. Staurosporine (1 μM) was the positive control for apoptosis. After Annexin V and PI staining, cells were analyzed by flow cytometry.

Results: Apoptosis was defined as Annexin V+ cells while necrosis was defined as PI+Annexin V- cells. Live cells were negative for both cell death markers. The positive control, Staurosporine, induced apoptosis in 80% of ACH-2 cells. Treatment with AZD5582 (100 nM) alone resulted in apoptosis in 7.7% and necrosis in 13% of ACH-2 cells. VTX (1 μM) treatment alone led to apoptosis in 10.7% and necrosis in 9.3% of ACH-2 cells. Combining AZD5582 and VTX increased apoptosis of ACH-2 cells to 51%, suggestive of a synergistic effect.

Conclusions: This in vitro data suggests the combination of VTX and AZD5582 may have a synergistic effect, increasing apoptosis of HIV-infected cells. Ongoing studies in CD4+ T cells from SIV-infected rhesus macaques treated with antiretroviral therapy aim to validate this. If the synergy of VTX and AD5582 is confirmed, this data will support testing this combination to deplete latent reservoirs in the rhesus macaque model in vivo.

Health-Related Quality of Life of Adolescent and Young Adult Pediatric Brain Tumor Survivors

Authors: Mekonnen, Rebekah; Ingerski, Lisa; Janss, Anna; and Mazewski, Claire

Presenting Author: Rebekah Mekonnen, BS

Poster Number: 5

Background: Existing literature supports the negative impact of a pediatric brain tumor (PBT) on long-term health-related quality of life (HRQoL); however, research regarding the HRQoL of adolescent and young adult (AYA) PBT survivors is scarce. Evaluating the effect of treatment-related factors is critical for promoting positive HRQoL outcomes in this unique population. It was hypothesized that AYA PBTs are more likely to experience negative HRQoL outcomes due to the intensity of treatment they receive.

Methods: Data were collected as part of a larger, IRB-approved, retrospective study of HRQoL in pediatric brain tumor survivorship. Self-reported and parent-proxy reported Pediatric Quality of Life Inventory (PedsQL) scores administered as part of standard clinical care were extracted from the electronic medical record. The PedsQL is a widely used HRQoL measure that has been reliability and validly used in pediatric oncology. Previously published minimal clinically important difference (MCID) values were used to define impairment across each individual PedsQL subscale and the total score.

Results: HRQoL subtest values were available for 39 PBTs (53.85% female, 13.68±2.40 years old at treatment initiation). Results suggested a percentage of AYAs experienced impaired HRQOL across both child- and parent-proxy reports respectively: 43.59% and 46.15% (Physical), 38.46% and 43.59% (Emotion), 38.46% and 46.15% (Social), 43.59% and 48.72% (School), 38.46% and 56.41% (Psychosocial), and 46.15% and 61.54% (Total) of AYA survivors fell in the impaired range. Additional multivariate analyses will investigate specific treatment-related factors (e.g., surgery, radiation, chemotherapy) related to impaired outcomes.

Conclusion: Results suggest that while many AYA PBT survivors do not demonstrate HRQoL impairments, there are a number of AYAs whose HRQoL falls below the MCID. Understanding potentially modifiable factors that could improve long-term outcomes for those PBT AYA survivors experiencing clinically significant impairments in HRQoL can aid in navigating their lives post-treatment in addition to maximizing the betterment of their well-being.

Exploring Partner Disclosure of Infertility Risk Among Survivors of Childhood Cancer: A Mixed Methods Approach

Authors: Pinkman, Kahyah; Klosky, James; Fitch, Kayla,; Rana, Shaheen; and Cherven, Brooke

Presenting Author: Kahyah Pinkman, PhD

Poster Number: 6

Background: Survivors of childhood cancer who received gonadotoxic treatment are faced with disclosing potential infertility to current/future romantic partners, which can cause distress. This study explored how fertility-related distress varied by partnership status and examined the relationship amongst sociodemographic variables and fertility-related distress.

Methods: Female cancer survivors (18-29y, <21y at cancer diagnosis) with prior gonadotoxic treatment were recruited from four cancer centers across the US to complete a web-based survey; a subset completed a qualitative interview. Fertility-related distress was assessed using the Partner Disclosure subscale of the Reproductive Concerns After Cancer measure (1-5, higher scores indicate greater distress). Multivariable linear regression was used to explore factors associated with distress; qualitative data were analyzed to identify themes related to partner disclosure.

Results: Among N=288 participants, mean age was 23.50±3.16 with 47.2% in a committed (married/living together/committed) relationship. Distress was significantly greater among single survivors compared with those in a committed relationship (3.16±1.33 vs. 2.59±1.34, p<.001). The overall regression model was significant ($F([5, 271]=8.67$, $p<0.001$), and indicated that compared with partnered survivors, single survivors had greater distress (p=.001) while those with greater certainty about fertility status (p=.002), who were older (p=.019), and who had public insurance (p=.003), endorsed less distress. Qualitatively, some participants reported concerns of perceived judgement or partners assuming their children will have cancer/birth defects. Disclosure concerns were also affected by perceptions of their current/future partner's family (i.e., traditional values, prioritization of biological grandchildren). Single participants described that the decision to disclose would be impacted by their partners' desires for future family (e.g., "how my partner feels about it").

Conclusion: Survivors of childhood cancer experience distress regarding disclosing potential infertility to future partners and could benefit from psychological support when navigating disclosure. Interventions focused on preparing survivors with strategies to navigate disclosure with future partners may be particularly beneficial.

Engineering Chemotherapy Resistant CAR-T Cells for Acute Myeloid Leukemia

Authors: Saleem, Nabil; Sullivan, Emily; and Raikar, Sunil

Presenting Author: Nabil Saleem, MD

Poster Number: 7

Background: Relapsed/refractory acute myeloid leukemia (R/R-AML) has extremely poor outcomes in children. While chimeric antigen receptor (CAR) T-cell immunotherapy has been successful in treating B-cell malignancies, targeting AML has proved to be more challenging due to tumor heterogeneity and on-target off-tumor side effects. Combining CAR-T immunotherapy with cytotoxic chemotherapy could potentially overcome these barriers. CAR-T cells can be genetically engineered to become chemotherapy-resistant, thereby enabling a combination chemo-cellular therapy approach. CD70 has high surface expression in AML, but low expression in non-malignant cells, making it an attractive AML-CAR target. Deoxycytidine kinase (dCK) is responsible for activation of cytotoxic nucleoside analogs cytarabine and fludarabine, drugs commonly used in R/R-AML. Knockdown of dCK expression results in significant resistance to both cytarabine and fludarabine and thus can be exploited as a strategy to create chemo-resistant CAR-T cells for AML. Our hypothesis is that the combination of chemo-resistant CAR-T cells and chemotherapy will be more effective than using either approach alone in treating R/R-AML.

Methods: CRISPR/Cas9 knockout of dCK expression in primary T cells followed by transduction with a lentiviral vector encoding the CD70-CAR construct will be used to generate chemo-resistant CAR-T cells for AML. dCK-edited CAR-T cells will be treated with cytarabine and fludarabine to determine whether dCK knockout confers chemotherapy resistance. We will then test the efficacy of dCK edited CAR-T cells in combination with fludarabine and/or cytarabine against three AML cell lines: MV4-11, Molm-13, and Nomo-1 using an in vitro flow cytometry-based cytotoxicity assay, followed by validation in in vivo xenograft models.

Results: We have tested four single guide RNAs (sgRNAs) to CRISPR knockout dCK in Jurkat T cells and are now quantifying knockout efficiency to select the best sgRNA for downstream studies. Additionally, we have successfully transduced Jurkat T cells with natural ligand and scFv based CD70-CAR lentiviral constructs. Using flow cytometry-based assays, we have confirmed cell-surface expression of these constructs as well as CAR-T cell activation when CD70-CAR transduced cells are co-incubated with AML target cells.

Conclusions: Our preliminary results show that generating dCK-edited CD70-CAR T cells is feasible, thus supporting the use of combination chemo-cellular therapy against R/R-AML.

Genetic Counseling in Cancer Survivorship Clinic

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Presenting Author: Erin Seibel, MMSc, LCGC

Poster Number: 8

Background: Childhood cancer survivors are more likely than their cancer-free counterparts to have a pathogenic germline mutation in a cancer predisposition gene (CPG). CPG mutations increase the likelihood of subsequent malignancies. Surveillance for CPG positive individuals can lead to earlier diagnosis, associated with improved prognosis and survival. Knowledge of a predisposition can help providers incorporate cancer surveillance into survivorship care. While there is a demonstrated utility for genetic counseling in survivorship clinics, many do not have genetic counselors (GCs), creating a barrier to genetic testing for patients. We hypothesize that embedding a GC in survivor clinic can facilitate testing to clarify more survivors' cancer risk and coordinate management to decrease mortality.

Objectives: The objective of this study is to evaluate the impact of full-time GC embedded in a cancer survivorship clinic.

Design/Method: This retrospective chart review analyzed the number of patients seen for genetic counseling, the number of tests completed, and actionable results received after GC integration in the Aflac Survivor Clinic on August 1, 2022. The percentage of patients seen in the first year with embedded GC coverage was compared to those seen between 8/1/21-7/31/22 when survivor patients were seen on a consult basis.

Results: One year prior to GC integration, 15.0% of survivors (144/960 visits) had engaged with genetic counseling (10.9% prior, 4.1% at survivor visit). After embedded GC coverage, 26.1% of patients (247/943 visits) engaged with genetic counseling – 15.4% cancer prior to their visit and 10.8% during their survivor visit. Since GC integration, genetic testing was completed for 76 patients. Of those, 48 had negative results, 18 (23.7%) had uncertain or carrier results, and 10 (13.1%) had positive results. Positive results included variants in the following genes: ETV6, WT1, TP53, RB1, CHEK2, and ATM. One patient was found to have a secondary malignancy shortly after testing, related to their CPG variant. All positive results have either pediatric management implications or cascade testing recommendations.

Conclusion: A GC embedded in a survivorship clinic increased the number of patients with GC contact by 75%, which can facilitate testing for patients and families, and identify predispositions to guide tailored cancer surveillance.

Integrated Electrical Conditioning and Dynamic Flow Perfusion for 3D Bioprinted Developing Human Heart Models

Authors: Fineman, Sarah; Jin, Linqi; and Serpooshan, Vahid

Presenting Author: Sarah Fineman, BS

Poster Number: 13

Background: The development of the human heart in embryos is a complex process susceptible to errors resulting in congenital heart defects (CHDs). Our research focuses on a severe CHD called Hypoplastic Left Heart Syndrome (HLHS), which has profound long-term complications despite postnatal surgical interventions. Research remains limited on the factors that contribute to HLHS. Due to the limitations of studying in vivo embryonic human hearts, a perfusable 3D human heart model at linear heart tube stage (day 22) was created by computer aided design (CAD) and 3D bioprinting. HLHS cardiomyocytes (CMs) were differentiated from human induced pluripotent stem cells (hiPSCs) and cultured in the 3D bioprinted heart models for studying cellular responses to the microenvironmental factor of flow hemodynamics and chronic external electrical conditioning. By increasing the microenvironmental accuracy for the embryonic heart model, our research works to uncover the factors that contribute to HLHS.

Methods: An idealistic human linear heart tube model was designed by CAD and 3D bioprinted via hydrogel based digital light processing (DLP). Customized 6-well plates were designed on CAD modeling software and 3D printed to facilitate the simultaneous delivery of dynamic flow perfusion and noninvasive electrical pacing. CM contractile function enhancement under chronic flow and pacing conditions over 5 days was evaluated through video-based contractile analysis obtained via brightfield microscopy. Immunofluorescence imaging was used to assess CM viability (cTnT) and expression of functional markers for voltage gated sodium channels (NaV1.5) and connexin 43 (CX43).

Results and Conclusions: The customized 6-well plate facilitated the simultaneous delivery of flow perfusion and pacing to CMs within a bioprinted scaffold. Significant differences in beats per minute (BPM), relaxation time, and beat rate variation were observed between pre and post chronic flow and pacing. Furthermore, there was no significant change in contractile stress. Immunofluorescence data was completed and markers for cTnT, NaV1.5, and CX43 were visualized within the constructs. By adding simultaneous mechanical factors to the bioprinted model, the findings of this study will contribute to better simulating the 3D microenvironment of the embryonic human heart development and understanding HLHS pathogenesis.

Targeting Mitochondrial Membrane Organization to Mitigate Energy Dysfunction in the Heart

Authors: Ghazal, Nasab; Huang, Benjamin; Park, Austin; Shoemaker, Luke; and Kwong, Jennifer Q

Presenting Author: Nasab Ghazal, MS

Poster Number: 14

Background: Mitochondrial dysfunction is a hallmark of heart failure, characterized by impaired oxidative phosphorylation (OXPHOS) and disrupted mitochondrial ultrastructure. The mitochondrial contact site and cristae organizing system (MICOS) complex plays a critical role in maintaining cristae architecture, which is essential for mitochondrial function. In the heart, loss of the mitochondrial phosphate carrier (PiC) disrupts ATP synthesis and triggers compensatory mitochondrial hyperproliferation, causing structural and functional decline. Meclizine, an FDA approved drug for vertigo, has been shown to promote metabolic adaptation, and thus, may ameliorate cardiac mitochondrial dysfunction. This study examines the effect of meclizine on cardiac function and mitochondrial structural defects in a PiC-deficient mouse model.

Methods: Cardiac-specific PiC knockout mice were treated with meclizine or vehicle, and cardiac function was assessed via echocardiography. Mass spectrometry-based proteomics was used to identify molecular changes associated with meclizine treatment. Mitochondrial assays were used to assess ATP synthesis, oxygen consumption rate, and metabolic shifts.

Results: Meclizine significantly improved cardiac function, increasing fractional shortening in PiC-deficient mice despite persistent mitochondrial dysfunction. Proteomics revealed that meclizine treatment causes an upregulation of subunits of the mitochondrial contact site and cristae organizing system (MICOS) complex, suggesting an impact on mitochondrial architecture. Additionally, meclizine reduced mitochondrial hyperproliferation, which may restore muscle organization and enhance contractility. While mitochondrial ATP synthesis and oxygen consumption remained impaired, the observed reduction in mitochondrial hyperproliferation and structural improvements point to a mechanism beyond energy production. Unexpectedly, glycolytic enzymes (PDK4 and LDH) were downregulated, with lactate levels decreasing, suggesting that meclizine can decrease lactic acidosis enhancing myocardial function without shifting to glycolysis.

Conclusion: Our data suggest that meclizine's ability to restore mitochondrial ultrastructure is due in part to the upregulation of the MICOS complex and reduction of mitochondrial hyperproliferation. In this way, meclizine may function by stabilizing mitochondrial architecture to improve cardiac muscle function. These results open new doors for therapeutic strategies to improve cardiac function by targeting mitochondrial organization and offer a new pathway to mitigate mitochondrial cardiomyopathies. Future research will aim to unlock the precise mechanisms by which mitochondrial structural changes influence cardiac health and explore the broader implications of heart disease treatment.

Sedating with Confidence: Hemodynamic and Respiratory Effects of Ketamine in Pediatric Cardiac ICU Patients

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Presenting Author: Khadija Haq, MD

Poster Number: 15

Background: In the pediatric cardiac intensive care unit (pCICU), procedural sedation must preserve cardiovascular stability. Ketamine is commonly used due to its rapid onset, dissociative effects, and generally favorable hemodynamic profile. However, data on its safety in children with complex cardiac disease remain limited. This study aimed to evaluate the safety and physiologic effects of ketamine during invasive line placement in pediatric CICU patients. To our knowledge, this represents the largest study to date examining ketamine use in this high-risk population.

Methods: We conducted a retrospective cohort study of patients aged 0–18 years who received ketamine for arterial or central venous line placement in a single quaternary-care pCICU. Sedation for intubation was excluded. Pre- and post-procedure vital signs were recorded, including heart rate, blood pressure, respiratory rate, and oxygen saturation. We also assessed respiratory support, inotropic use, and adverse events within 24 hours of ketamine administration (e.g., intubation, arrhythmia, cardiac arrest, ECMO, or death). The Wilcoxon signed-rank test was used to analyze changes in vital signs.

Results: We included 285 patients across 293 sedation encounters. The median age was 2.4 months (IQR: 0.75–5.6), with most patients being infants with complex congenital heart disease. Heart rate decreased slightly post-ketamine (147 to 146 bpm, $p = 0.001$). Systolic blood pressure remained stable (85.8 vs. 86.2 mmHg, $p = 0.742$), while diastolic pressure showed a modest decline (49.6 to 49.1 mmHg, $p = 0.030$). Oxygen saturation increased slightly (92.0% to 92.5%, $p = 0.025$), and respiratory rate declined (31.9 to 28.7 breaths/min, $p = 0.019$), neither of which was clinically significant. No deaths occurred. Intubation occurred in 18% of cases, likely reflecting baseline acuity. Arrhythmias (1%), cardiac arrest (1.4%), and ECMO (0.3%) were rare.

Conclusion: Ketamine use for procedural sedation in the pCICU was associated with minimal hemodynamic and respiratory changes and low rates of serious complications. These findings support its safety in this high-risk population and underscore the need for further research comparing sedative agents and standardizing protocols.

Metabolic and Mitochondrial Dysfunction in CDKL5 Deficiency: A Seahorse XF Analysis

Authors: Liporace, Eliana; Zlatic, Stephanie; and Faundez, Victor

Presenting Author: Eliana Liporace

Poster Number: 17

CDKL5 Deficiency Disorder (CDD) is a severe neurodevelopmental disorder caused by mutations in the CDKL5 gene, characterized by early-onset epilepsy, intellectual disability, and motor impairments. While CDD shares clinical features with Rett syndrome (RTT, MECP2-linked), its molecular underpinnings are distinct. Mass spectrometry (MS) of cerebrospinal fluid (CSF) from Cdkl5−/y and Mecp2−/y mice revealed divergent metabolic pathologies: CDD CSF exhibited enrichment in mitochondrial inner membrane pathways (GO:0005743, p = 7.5e−38), marked by downregulated respiratory complex I subunits (Ndufa12, Ndufs4) and solute carriers (Slc25a11, Slc25a18). In contrast, RTT CSF proteomes implicated synaptic vesicle cycling (GO:0016079) and fatty acid oxidation (Acadm, Cpt1a), with no overlap in CDD-specific mitochondrial signatures.

To investigate these metabolic disruptions, we analyzed CDKL5 knockout Hap1 cells (KO1: exon 6 22bp deletion; KO9: exon 5 10bp deletion) using Seahorse XF assays. Under glucose, KO cells showed elevated basal respiration (+42%, $p<0.01$) but impaired maximal respiration (−42%) and ATP production (−40%), alongside increased proton leak (+67%). Glutamine supplementation partially rescued ATP synthesis (KO1: +28%, KO9: +32%) but failed to restore maximal respiration, reflecting compensatory reliance on anaplerosis. Proteomics linked this to downregulated mitochondrial pyruvate carriers (MPC1/2, 2.1-fold, $p<0.05$) and upregulated glutaminolysis (Gls1, 1.8-fold), highlighting CDD's substrate prioritization defects.

These findings establish CDD as a disorder of mitochondrial substrate shuttling, driven by complex I dysfunction and metabolic inflexibility. Future studies will target mitochondrial carrier system (MCS) redundancy, testing alternative fuels (e.g., fatty acids, ketones) and pharmacological MPC reactivation to restore bioenergetic homeostasis. By delineating CDD-specific pathways distinct from RTT, this work advances precision therapeutic strategies for neurodevelopmental disorders.

Investigating the Role of Placental Development in Slc25a1-Associated Congenital Heart Disease

Authors: Liu, Ethan H.; Ohanele, Chiemela; Park, Austin S.; and Kwong, Jennifer Q.

Presenting Author: Ethan Liu, BS

Poster Number: 18

Background: Congenital heart disease (CHD) is a leading cause of neonatal mortality, accounting for 11% of stillbirths and approximately 35% of infant deaths. 22q11.2 deletion syndrome, a chromosomal microdeletion, is a leading genetic cause of CHDs with approximately 60-80% of affected individuals experiencing CHDs. We identified Slc25a1, a mitochondrial citrate carrier gene found within 22q11.2DS critical deletion region, as required for cardiac morphogenesis in embryonic mice due to its regulation of critical metabolic transitions in the embryonic heart. Interestingly, Slc25a1 is haploinsufficient, with congenital heart defects occurring in hemizygous deleted embryos, suggesting a cardiac intrinsic role for Slc25a1 in cardiac development. However, other groups suggest that Slc25a1 deletion-associated congenital heart defects are due to loss of Slc25a1 in the placenta, as the placenta-fetal heart axis, particularly the placental trophoblasts, play a critical role in regulating oxygen and nutrient exchange required for embryonic heart development. While CHDs were observed in our Slc25a1+/- and Slc25a1-/- hearts, little is known about the role of the placenta in mediating CHDs observed in those animals. Our research investigates whether placental abnormalities contribute to the CHDs observed in Slc25a1+/- and Slc25a1-/- embryos. We hypothesize that Slc25a1-dependent placental defects play a critical role in CHDs observed in Slc25a1 deficient mice.

Methods: We performed timed matings of Slc25a1+/- mice, sectioned, performed histological analysis on placentas from Slc25a1+/+, Slc25a1+/-, and Slc25a1-/- embryos, and quantified thicknesses of placental layers where trophoblast cells are enriched: the junctional zone and labyrinth.

Results: Placentas from Slc25a1+/- embryos showed no significant differences in junctional zone and labyrinth to whole placenta ratio when compared to Slc25a1+/+ controls. Placentas from the Slc25a1-/- embryos showed significantly decreased junctional zone to whole placenta ratio and an increased labyrinth to whole placenta ratio compared to Slc25a1+/+ controls.

Conclusions: These data suggest that the role of Slc25a1 in regulating placental development is not required for cardiac morphogenesis in Slc25a1+/- embryos. In contrast, the enlarged labyrinth observed in Slc25a1-/- placentas may represent a compensatory adaptation to the reduced junctional zone. Furthermore, extra-placental tissues must be considered when exploring the role of Slc25a1 in congenital heart disease.

Bile Acids as Immunomodulatory Signals in Cystic Fibrosis

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Presenting Author: Samantha Durfey, PhD

Poster Number: 20

Background: Over the past decade, CFTR modulators have revolutionized the treatment of cystic fibrosis (CF). Despite significant improvements in disease morbidity and mortality, chronic lung infections persist in people with CF (pwCF) after they begin modulators. Exposure to CF airway fluid reduces bacterial phagocytosis and killing in healthy macrophages, which suggests abnormal tolerance signals exist in the airways of pwCF. We propose bile acids as a contributing tolerance signal, as bile acids are dysregulated in the CF airway and can influence gut and liver immune cell function in healthy people. However, it is unknown whether healthy or CF airway macrophages respond to bile acids and if bile acids remain dysregulated after CFTR modulators.

Methods: Single cell RNA sequencing data from the Chan-Zuckerberg CellxGene database was mined to determine if bile acid receptors are expressed in lung-resident macrophages. Human macrophages were exposed to bile acids ex vivo, and bile acids' effects on bacterial growth and macrophage cell death were determined. Metabolomics (LC/MS followed by analysis with Compound Discoverer) was performed on plasma from pwCF before and after starting the CFTR modulator, elexacaftor/tezacaftor/ivacaftor (ETI).

Results: We found monocytes and macrophages in the lung and liver express bile acid receptors at similar levels. Plasma metabolomics revealed that two bile acids (glycocholic acid and glycochenodeoxycholic acid) are amongst the top 10 most differentially abundant molecules in pwCF compared to healthy people, and both remain elevated after ETI. Indeed, glycocholic acid was the second most differentially abundant molecule before and after ETI. Preliminary results revealed bile acids were not toxic towards bacterial CF pathogens (Pseudomonas aeruginosa, Staphylococcus aureus, and Burkholderia cenocepacia), but macrophage cell death was enhanced in the presence of deoxycholic acid.

Conclusion: We demonstrated the potential for bile acids to influence macrophage function in modulator-treated pwCF. Lung macrophages express bile acid receptors, and bile acids are amongst the most differentially abundant molecules in CF, with persistent aberrations following modulator therapy. Ongoing work will assess the effects of bile acid exposure on bacterial killing by patient-derived macrophages in a coordinated gut-lung axis study.

Evaluating the Efficiency of Air-liquid Interface (ALI) as an In Vitro Model for Studying Viral Respiratory Infections in Cystic Fibrosis

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Presenting Author: Julia LeCher, PhD (presenting for Cameron Mahanke, BS)

Poster Number: 21

BACKGROUND: Cystic fibrosis (CF) is a genetic disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, and symptoms primarily manifest in the lungs and pancreas. CF is one of the most common genetic diseases among children, and early intervention is crucial for slowing disease progression and increasing life expectancy. To study viral respiratory infections, we need an in vitro model that closely resembles the CF lung environment. Air-liquid interface (ALI) is a 3D culturing approach utilizing primary human respiratory cells that mimics the in vivo environment of human airway epithelium and can be applied to studying viral infections and antiviral drug development in CF patients.

METHODS: Primary human basal bronchial/tracheal epithelial cells from healthy donors and CF donors were obtained commercially. Cells were expanded in basal media and then transferred to collagen-coated transwells (0.4 µm polyester membrane) in 24-well plates. To establish ALI, media was removed from the apical chamber and ALI-differentiation media was added to the basolateral side of the transwell chamber on day three. Media was changed in the basolateral chamber every 2 - 3 days, and Trans-Epithelial Electrical Resistance (TEER) was tested weekly using an epithelial volt-ohm meter and calculated by: TEER = Ωsample - Ωblank * 0.33 cm^2.

RESULTS: After 3 - 4 weeks, cultures showed robust mucus secretion and beating cilia, indicating the formation of a mucociliary environment. TEER values of ≥ 250 Ω/cm^2 validated the presence of a polarized membrane. We found differences between the healthy and CF donors regarding the mucociliary environment. In addition, the CF donor cells had lower TEER values than the healthy donor cells, indicating decreased barrier function.

CONCLUSIONS: Mucus formation, beating cilia, and the formation of a polarized membrane demonstrate that ALI is a useful 3D model that mimics the in vivo environment of human lungs. The phenotypic difference between CF and non-CF donors supports ALI is a valuable model for studying CF. We are currently using the ALI model to evaluate differences in infection and replication kinetics of respiratory viruses between healthy and CF donors. We are also evaluating how CF-specific therapeutics may impact infection outcomes.

Metabolomics Profiling in Pediatric Cystic Fibrosis Before and After Modulator Therapy Compared with Healthy Controls

Authors: Maaskant, Jessica; Durfey, Sam; Collins, Genoah; Chandler, Joshua; and Kopp, Benjamin

Presenting Author: Jessica Maaskant, MS

Poster Number: 23

Background: Cystic Fibrosis (CF) is a genetic disorder affecting multiple organ systems and ~40,000 individuals in the United States. The introduction of the modulator therapy Elexacaftor/Tezacaftor/Ivacaftor (ETI), which improves the function of the defective CFTR protein, has significantly improved outcomes for people with CF (pwCF). However, ~10% of pwCF are not eligible for ETI and even those eligible do not respond equally. The extent to which ETI alters the metabolic profile of pwCF and the role of metabolism in predicting treatment responses remains unclear.

Methods: We characterized plasma metabolic profiles of healthy donors and teen and adult pwCF at baseline and 3 months after ETI initiation. Metabolomics analysis quantified over 1700 metabolites, with 225 high-confidence metabolites identified per sample using high resolution mass spectrometry. Metaboanalyst identified significantly different metabolites (FDR $p<0.05$ and FC >2).

Results: There were 39 CF and 38 healthy donors. Metabolomics identified unique altered metabolite patterns pre- and post-ETI within pwCF and compared to controls. Of these metabolites, trans-3-indoleacrylic acid and piperine were decreased and N-a-acetyl-lysine were uniquely elevated in pwCF pre-ETI. Trigonelline, hypoxanthine, and inosine were persistently the most significantly decreased metabolites in pwCF even after modulator treatment. Pathways altered by ETI therapy included bile acid signaling and aromatic amino acids involved in protein synthesis. There were no significant changes in metabolic profiles when stratified by sweat chloride concentrations.

Conclusions: CFTR modulators modestly impacted plasma metabolic profiles in pwCF. Further investigation is warranted to understand how CFTR regulates metabolic signaling and persistent derangements in pwCF. Ongoing analyses will determine if metabolic differences are associated with clinical outcomes post modulator therapy.

Red Blood Cell-derived Nanoparticles as Drug Carriers to Human Airway Neutrophils in Cystic Fibrosis

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Poster Number: 26

Background: In cystic fibrosis (CF), the airway neutrophils undergo transcriptional reprogramming resulting in the upregulation of anabolism and active suppression of antimicrobial activity, which underly bacterial tolerance in CF airways. Small molecules that treat these maladaptive responses are being developed to decrease neutrophilic inflammation, but current delivery methods do not adequately target neutrophils. We propose using red blood cell (RBC)-derived nanoparticles (RBCNPs) as drug carriers, leveraging their natural, evolved affinity for and compatibility with scavenger cells (notably neutrophils) to maximize cargo delivery to these cells while eschewing immune activation typically seen with artificial lipid nanoparticles.

Methods: RBCNPs are generated by soft extrusion and loaded with a therapeutic cargo using hypotonic hemolysis followed by isotonic resealing. Core methods for RBCNP characterization include nanoparticle tracking analysis (NTA) for particle size and concentration, electron microscopy (EM) for morphology, and spectrophotometry to measure delivery efficacy. We selected the JAK/STAT inhibitor, baricitinib, as a model compound to measure delivery efficiency due to its defined effect on immune pathways, notably interferon (IFN) signaling. As model recipient cells, we used HEK-blue IFN α/β cells which induce expression of secreted embryonic alkaline phosphatase (SEAP) when stimulated with type I IFN.

Results: The soft extrusion method produced a highly homogenous population of RBCNPs suitable for cargo loading. NTA demonstrated (100-400) nm sized, high yield RBCNPs and reproducibility consistent with the controlled procedure of soft extrusion. EM analysis confirmed high population homogeneity of RBCNPs, as well as the presence of mother RBCs before extrusion and their transformation into NPs after extrusion. To test delivery efficacy, baricitinib-loaded RBCNPs were administered to HEK-blue IFN α/β cells and detection of SEAP was confirmed by spectrophotometry, showing a quantifiable effect of the delivered drug.

Conclusions: Our results demonstrate the potential of RBCNPs as natural, easily manufactured, and efficient drug carriers able to alter immune signaling when loaded with relevant drugs. Subsequent work will assess delivery to airway neutrophils, as a novel approach to mitigating chronic inflammation and enhancing bacterial clearance in CF.

Partial Inhibition of Proteins in Distinct Structural Regions of the Ribosome Confer Differential Rescue of Cystic Fibrosis-Causing Nonsense Variants

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Poster Number: 27

Background: Cystic fibrosis (CF) is a lethal, autosomal recessive disorder caused by mutation of the CF transmembrane conductance regulator (CFTR). Among the U.S. CF population, ~13% of patients encode CFTR premature termination codons (PTCs) – or "nonsense" variants – the most prevalent of which are G542X and W1282X. These variants confer unstable transcripts and truncated proteins, and remain without an effective therapeutic intervention. Our work focuses on elucidating mechanistic impact and therapeutic potential of genetic modifiers that influence PTC biogenesis. We previously modeled CFTR in a homologous yeast system to ascertain gene-gene interaction networks responsible for read-through of CF-causing PTCs. High-throughput analysis revealed several gene deletions, such as ribosomal proteins L12 (RPL12/uL11) and L8 (RPL8/uL2), that significantly improved PTC functional expression.

Methods: For the present study, differential effects of RPL12 or RPL8 depletion elicited on CFTR PTCs were determined with siRNA knockdown using Fischer rat thyroid cells, CF bronchial epithelia, and primary human nasal epithelia. Immortalized cells were stably transduced with G542X- or W1282X-CFTR cDNA. Additional isogenic lines were engineered to encode an in-frame C-terminal NanoLuc reporter to measure PTC read-through, or a horseradish peroxidase tag to detect CFTR plasma membrane (PM) localization. Cell viability, CFTR processing, and channel function were monitored. Tests of additivity/synergy were conducted with G418 (read-through agent) or clinically approved CFTR modulators, elexacaftor-tezacaftor-ivacaftor (ETI).

Results: Partial silencing (~50%) of RPL12 or RPL8 significantly enhanced W1282X and G542X PM density. W1282X trafficking was augmented in a multiplicative manner by combination of ETI with siRPL8 or siRPL12. PM localization of G542X-CFTR was greatly improved with siRPL12 or siRPL8. RPL12 suppression robustly augmented full-length protein production for both G542X and W1282X. In addition, W1282X open channel probability was increased by inhibiting RPL8 or RPL12. Knockdown of either ribosomal protein significantly increased W1282X short-circuit currents.

Conclusions: Our results indicate partial inhibition of RPL8 or RPL12 should be considered as a novel therapeutic strategy for nonsense suppression, the effects of which may be synergistically augmented by combination with CFTR modulators. This work will inform future studies designed to examine feasibility, efficacy, and tolerability of targeting ribosomal 'hits' in CF cells and animal models.

Surgery First in Pediatric Hemolytic Disease: A Smarter Approach to Choledocholithiasis?

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Poster Number: 29

Background: Hemolytic disease is the second most common cause of biliary stones in the pediatric population. A surgery-first (SF) approach, utilizing laparoscopic common bile duct exploration (LCBDE) when needed, has demonstrated improved outcomes compared to endoscopic retrograde cholangiopancreatography (ERCP) first (EF) in children with choledocholithiasis. SF and EF have not been directly compared in children with hemolytic disease. Given the higher surgical, procedural, and anesthetic risks in this patient population, we aimed to evaluate the outcomes of SF vs EF in this cohort.

Method: We conducted a multi-institutional retrospective review of patients <18 years of age diagnosed with choledocholithiasis between 2012 and 2025. Demographic data, clinical characteristics, and outcomes were recorded. We compared SF vs EF in the hemolytic disease population and compared outcomes of the hemolytic to non-hemolytic cohort. Wilcoxon rank sum test was performed on continuous variables, and Pearson's Chi-squared test and Fisher's exact test were used to analyze categorical variables, with a significance level of 0.05.

Results: Among 438 children with choledocholithiasis, the majority were females (71.7%), with a median age of 15.2 years (IQR: 13.10, 16.80). Of these, 54 had a hemolytic disease, including HbSS (55.6%), hereditary spherocytosis (27.8%), HbSC (7.4%), HbSS/BO (3.7%), beta thalassemia trait (1.9%), hemoglobin E beta zero thalassemia (1.9%) and Hb-Mainz hemolytic anemia (1.9%). Patients with a hemolytic disease were younger, with a lower BMI, more likely to be male, and less likely to have cholecystitis. Patients with hemolytic diseases had a shorter operative time than those without were more likely to return to the emergency department (14.8% vs 5.5%, p=0.017). Within the hemolytic diseases' cohort, patients treated with a SF approach had a shorter length of hospital stay (2 days vs 6 days, p=0.009).

Conclusion: A surgery first approach, with laparoscopic common bile duct exploration, when necessary, is effective in the management of choledocholithiasis in children with hemolytic disease. The shorter length of hospital stay with the surgery first approach in children with choledocholithiasis is also demonstrated in the hemolytic diseases' population, without increased complications.

Exploring the Immunogenicity of Recombinant FVIII in C57BL/6 Mice: A Pathway to Acquired Hemophilia A Modeling

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Presenting Author: Sumaiya Dickens, BS

Poster Number: 31

BACKGROUND: Hemophilia A is a rare genetic bleeding disorder caused by deficiency or dysfunction of coagulation Factor VIII (FVIII). Although current treatment relies on repeated infusions of recombinant FVIII (rFVIII), a major complication is the development of inhibitory antibodies, which occur in approximately 20–30% of patients with severe Hemophilia A. These antibodies neutralize FVIII activity and severely limit treatment efficacy. In preclinical studies, C57BL/6 mice are commonly used as controls in Hemophilia A research. It is known that these mice produce IgG antibodies against human rFVIII following repeated exposure; however, it remains unclear whether these antibodies cross-react with and target the mice's endogenous FVIII. Given the high degree of homology between human and murine FVIII, we hypothesized that repeated injections of rFVIII in C57BL/6 mice may lead to the development of autoantibodies against native murine FVIII.

OBJECTIVE: To determine whether C57BL/6 mice can serve as a viable model for acquired Hemophilia A, providing insights into the immune responses that contribute to inhibitor development.

METHODS: Protein sequence alignment of human and murine FVIII was performed using UniProt data and NCBI BLASTp to assess domain-specific and full-length homology. The average sequence identity across major structural domains (A1, A2, B, A3, C1, C2) was 82.15%, while full-length sequence identity, including disordered regions, was 72.37%. C57BL/6 mice (n=8) were randomly divided into human recombinant FVIII-treated and saline control groups. Mice received three weekly retro-orbital injections of 2 µg rFVIII or saline. Retro-orbital blood collection was performed six days post-injection, and plasma was isolated and stored at –80°C. Anti-human FVIII IgG and IgM levels were quantified weekly by ELISA. Clotting activity in response to exogenous rFVIII was assessed using an aPTT-based one-stage clotting assay. To evaluate the development of autoantibodies against endogenous murine FVIII, Bethesda assays were performed on post-treatment plasma using mouse-derived FACT as the FVIII source. Standard curves for FVIII activity were generated via serial dilution of human and mouse FACT in HBS-Tween buffer and tested in FVIII-deficient plasma.

RESULTS: Standard curves generated using both human and mouse FACT yielded similar clotting times, with R^2 values of 0.99, confirming assay reliability. ELISA results showed that mice injected with human rFVIII developed significantly elevated anti-human FVIII IgG and IgM levels after three weekly injections. Correspondingly, clotting assays demonstrated a significant reduction in human FVIII activity, indicating the development of inhibitory anti-FVIII antibodies. However, Bethesda assays revealed no detectable inhibitory titers against endogenous murine FVIII, suggesting a lack of autoantibody formation.

Characterization of Factor VIII Specific B Cell Subsets in Mice

Authors: Juarez Rojas, Silvia and Doshi, Bhavya

Presenting Author: Silvia Juarez Rojas, BA

Poster Number: 32

Background and Objective: Hemophilia A (HA) is a severe congenital bleeding disorder resulting from a deficiency of coagulation factor VIII (FVIII). Treatment with FVIII protein replacement, to prevent bleeding, results in anti-FVIII antibodies ("inhibitors") in ~30% of patients with severe HA, limiting hemostatic options for breakthrough bleeding. FVIII immune tolerance induction (ITI), involving years of daily high dose intravenous FVIII infusions, is cumbersome, carries risk of complications, and is unsuccessful with inhibitors titers over 200 BU. Understanding the contribution of FVIII-specific memory and plasma (PC) B cell subsets in FVIII immune responses may help inform ITI regimen choice, as maturing titers may indicate generation of long-lived plasma cells (LLPCs), which are difficult to eradicate with ITI alone (as it targets CD20+ memory cells). Here, we aimed to delineate the contribution of FVIII-experienced plasma and memory B cells in the FVIII immune response.

Methods: An AICDA Cre/Lox model that expresses YFP when germinal center B cells respond to antigen was immunized with FVIII weekly x 6. Splenic and bone marrow memory and plasma cells, including subsets, were quantified by flow cytometry. FVIII-specificity of B cells was confirmed with labeled FVIII. Inhibitor titer and anti-FVIII IgG were measured by Bethesda and ELISA assays, respectively. Data were compared by Mann-Whitney U, ANOVA, and Pearson correlation.

Results: In the spleen, inhibitor positive mice showed enrichment of FVIII+ PCs (6.5-fold, $p<0.001$), memory B cells (2.8-fold, $p<0.01$) and plasmablasts (3.3-fold, $p<0.01$). In the bone marrow, inhibitor positive mice demonstrated enrichment of FVIII+ LLPCs (2.1-fold, $p<0.05$) and memory B cells (3.5-fold, $p< 0.001$). Mice with high titer inhibitors (> 250 BU), had higher splenic PCs and marrow LLPCs compared to non-inhibitor mice whereas mice with intermediate titers (50-250 BU) had expansion of splenic and marrow memory, but not plasma, B cells.

Conclusions: These data demonstrate that FVIII+ PCs reside in both the spleen and bone marrow. The expansion of bone marrow LLPCs with high titers may hinder tolerance attempts with routine ITI and suggest need for plasma cell targeted immune modulation. Further human correlation of these data could help tailor ITI regimens in HA patients.

Procalcitonin Levels in Hospitalized Children with Sickle Cell Disease (SCD) During Acute Vaso-Occlusive Pain Episodes (VOE)

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Presenting Author: Giorgi Maziashvili, MD

Poster Number: 33

Procalcitonin (PCT) is a marker of systemic inflammation that helps distinguish bacterial infection from other sources of fever. Given its elevated levels during serious bacterial infections(SBI), standardized PCT cutoff levels are commonly used to guide antibiotic stewardship. However, differentiating the source of fever during SCD-VOE remains challenging.

Our aim is to assess the plasma PCT levels in patients hospitalized for SCD-VOE and its association with various clinical and laboratory parameters, including fever and acute chest syndrome(ACS).

We conducted a secondary analysis of samples from a pharmacokinetics/pharmacodynamics study and Phase-2 randomized controlled trial of IV arginine therapy in patients aged 3-21 years with SCD-VOE. Plasma PCT was measured at emergency department (ED) presentation & hospital discharge(DC). Non-SCD SBI studies classify PCT levels ≥0.5ng/mL as elevated and ≥2ng/mL as high risk. Associations between clinical/lab variables and PCT were analyzed.

The study included 102 patients (mean age 13±4 years; 49% male; 66% HbSS genotype; 68% receiving hydroxyurea). There were no differences in patient demographics or clinical/laboratory parameters. Fever ≥38.0°C was present in 14% of patients at ED presentation, while 18% developed fever during hospitalization. Three patients were diagnosed with ACS at ED presentation and eight developed ACS during hospitalization. Mean PCT at presentation was (1.2±0.8 ng/mL) with PCT ≥0.5ng/mL in 69% of patients, among which 9% had PCT ≥2ng/mL. Although PCT levels remained ≥0.5 at discharge in 60% of patients, levels dropped significantly between ED presentation and discharge (1.2±0.8 vs 0.9±0.8, p=0.002). No correlation between PCT and arginine levels was noted.

Plasma PCT is elevated in children with SCD, likely reflecting systemic inflammation that persists at discharge and is unrelated to bacterial infection. PCT≥0.5 did not correlate with fever, ACS, chest pain, white blood cell count, or other assessed clinical/laboratory parameters. Notably, no patients in this cohort had SBI. While PCT shows promise for antibiotic stewardship in healthy populations, its predictive value in SCD require more research. Although the role of PCT to identify SBI risk in SCD is unclear, PCT levels<0.5ng/mL may suggest low risk, potentially allowing for antibiotic stewardship in the future.

Storage-induced Microerythrocyte Content in Red Blood Cell (RBC) Products

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Presenting Author: Zahra Naseh, MD

Poster Number: 34

Background: Pediatric patients with sickle cell disease (SCD) often require frequent and life-saving red blood cell (RBC) transfusions. The response to each transfusion is variable, with little known about the factors influencing transfused RBC lifespan. During cold storage, donor RBCs experience molecular and morphologic alterations, known as the storage lesion, which may impact RBC survival and compromise transfusion efficacy. Our study aims to evaluate morphologic changes of stored RBCs and development of storage-induced micro-erythrocytes (SMEs).

Methods: We employed carboxy-fluorescein-succinimidyl-ester (CFSE), a fluorescent marker used to track cell proliferation, which has previously been shown to be high in SMEs. RBCs were obtained from residual tubing from units transfused to patients with SCD. RBC concentrates (0.3125 to 20 million/ml) were prepared then stained with CFSE dye at different concentrations (1.56 to 200.0 nM) and incubated at 37°C for 16 to 48 hours. Flow cytometry was utilized to measure CFSE concentration, cell density, and to identify and quantify SMEs. Imaging flow technology and electron microscopy were used to visualize CFSE-high and CFSE-low RBC populations and confirm SME morphologic changes.

Results: We tested 48 RBC samples from 24 RBC units at time points that ranged from 15-70 days from initial donor collection. CFSE concentration, cell density, and incubation time impacted bimodal separation of low vs. high CFSE-RBCs. Maximum separation of RBCs was observed when using a CFSE concentration of 50 nM with incubation for 48 hours on RBC concentrates with 0.625-5.5 million/ml range.

The percentage of SMEs in each RBC unit ranged from 18% (in 15-day-old units) to 37% (in 42-day-old units). Among 16 RBC units tested, the SME concentration was highly correlated to RBC product age ($r=0.91$, $p<0.0001$).

Finally, imaging flow and electron microscopy confirmed SME morphologic changes in high-CFSE RBC populations.

Conclusion: Flow cytometry provides an effective tool for quantifying storage-induced alterations of RBCs. This study underscores the importance of monitoring RBC quality throughout storage to enhance transfusion outcomes and address concerns related to the efficacy of stored blood. Future studies will evaluate SME percentage present in biotin-labelled RBC units transfused to patients with SCD and evaluate potential impacts on RBC survival.

Childhood Anemia Initiative at Ethnē Health Community Clinic

Authors: Fletcher, Lydia*; Vo, Sophie*; Stewart, Kimberly; and Boden, Laurie

Presenting Authors: Sophie Vo, BS, BA and Lydia Fletcher, BS

Poster Number: 35

Background: Clarkston, GA is home to a vibrant refugee community, with residents from over 40 different countries. The refugee population is particularly impacted by childhood anemia, with an estimated prevalence of 36.54 among refugee children globally. We worked with Ethnē Health Community Clinic to address the growing frequency of iron deficiency discovered during well child visits. The aim of our project was to investigate the dietary habits of Clarkston's residents, identify potential interventions, and evaluate the feasibility of current dietary recommendations. Ultimately, our goal was to create a culturally sensitive educational tool to assess and recommend steps to improve a child's current iron intake.

Methods: We conducted home nutrition visits for five families from Afghan, Karen, and Eritrean backgrounds. We gathered observations about their meals and most frequented grocery stores to identify accessible, iron-rich foods that could be incorporated into each culture's diet. The intervention focused on iron-deficiency anemia, the most prevalent form of nutritional deficiency in the pediatric population at Ethnē. The first part of the initiative consisted of making an interactive pamphlet of iron-rich grains, vegetables, meat, fruits, and nuts that families could circle. A detailed breakdown of the iron content for one, ½, and ¼ cup of each food item was provided so that physicians could calculate patients' iron intake. The second part of our initiative involved creating a compendium of pictures from local grocery stores of iron-rich food items and providing familiar examples of the food items listed in the pamphlet.

Results: Personalized, culturally sensitive nutrition education better equips families with the knowledge to ensure their children have adequate iron intake. Pamphlets enhanced nutrition assessment and education by providing a quick estimate for iron intake. Ethnē is a site for the Community Learning Social Medicine longitudinal course at Emory SOM, so the nutrition initiative will be continuously assessed and refined.

Conclusion: Clarkston is a diverse community encompassing a wide variety of cultural backgrounds. Consequently, it is important to understand each patient's culture before suggesting dietary interventions. Conducting home visits gave us insight beyond patient lab values into accessible and desirable iron-rich foods.

Impact of Freeze-thaw Cycles on Detection on Influenza A and B Virus When Assessing Novel Multiplex Diagnostic Assays

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Presenting Author: Heather Bowers, BS

Poster Number: 38

Background: As a part of the NIH's Rapid Acceleration of Diagnostics (RADx) program, viral panels are being used to evaluate new rapid antigen tests (RATs) for their sensitivity and efficacy. Many of these newly emerging RATs are capable of the simultaneous detection of FluA, FluB, and SARS-CoV-2. These highly validated panels often shipped to outside laboratories and may intentionally or unintentionally undergo a freeze-thaw cycle during transit. To ensure the efficacy of these panels is maintained at all times, we characterized the effect of four freeze-thaw cycles on FluA and FluB serial dilutions using RT-qPCR, two RATs and an already commercially available point-of-case (PoC) assay.

Methods: Viral stocks of live Influenza A and B virus were obtained from BEI. A serial dilution of the virus made in negative nasal wash (Lee BioSolutions) was prepared and aliquot into separate panels. The serial dilutions were tested on the two rapid antigen tests (RATs) and the point-of-care assay fresh and after experiencing one, two, three, or four freeze thaws. Testing of the two RATs was done in triplicate in a limit of detection (LOD) range-finding experiment. The LOD is determined to be the last dilution where all three replicates were positive. The results of the RATs and PoC assay were compared for the different freeze-thaws to observe efficacy.

Results: While there were minor variations in Ct values for both FluA and FluB throughout the freeze-thaw cycles, both the RATs and the PoC assay were able to successfully detect up to four freeze thaw cycles without losing efficacy.

Conclusion: Correct handling of these viral panels which are used to evaluate emerging rapid antigen test (RATs) is critical for correctly determining the sensitivity and efficacy. While in transport, storage conditions are challenging to maintain and must be carefully considered by laboratories and engineers designing or validating diagnostics tests. Luckily, it seems are though there may be some leniency when it comes to freeze-thaws cycles on live influenza A and B virus.

Barriers to Timely and Accurate Follow-up Testing for Abnormal SCID Newborn Screening

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Presenting Author: Jacqueline Comiter, BA

Poster Number: 39

Background: Abnormal Severe Combined Immunodeficiency (SCID) newborn screening (NBS) results require follow-up testing with repeat TREC PCR or lymphocyte subset test with CD45RA/RO for definitive diagnosis and timely treatment. However, delays in or errors obtaining follow-up testing can delay diagnosis and treatment of SCID.

Methods: The Georgia SCID NBS follow-up database from 2019-2023 was analyzed to determine the timeliness and accuracy of follow-up testing after an abnormal SCID NBS. A quality improvement project was then planned to address barriers to follow-up testing.

Results: From 2019-2023, 7,210 abnormal SCID NBS results required follow-up testing with either repeat TREC PCR or lymphocyte subset test with CD45RA/RO. When follow-up testing was indicated, the mean time between initiation of follow-up and the date repeat screening was obtained was 6.5 days. 4.8% of results required follow-up testing with lymphocyte subset test with CD45RA/RO, specifically. 13% of follow-up tests with lymphocyte subset test with CD45RA/RO were performed incorrectly. 79% of incorrectly performed lymphocyte subset tests were incorrect because they were missing CD45RA/RO. Other reasons for incorrectly performed lymphocyte subset tests include incorrect panel orders (e.g., leukemia, SCID gene, paroxysmal nocturnal hemoglobinuria, and Lyme disease panels), delayed collection, and collection that never took place.

Conclusions: Over 10% of SCID NBS follow-up testing with lymphocyte subsets was performed incorrectly from 2019-2023 in Georgia. The previous recommendation page faxed to offices included general background information followed by lab-specific ordering instructions. This document used the wording “flow cytometry,” included instructions for labs that did not actually offer CD45RA/RO testing, and did not provide ordering instructions for Quest. Requiring all infants to have a lymphocyte subset with CD45RA/RO was found to be a potential barrier to timely and accurate follow-up in a 2021 study of 10 years of NBS for SCID in Massachusetts. Thus, the recommendation page was updated to minimize the number of incorrectly performed lymphocyte subset tests by removing the requirement for CD45RA/RO, omitting the words “flow cytometry” to avoid confusion with leukemia/lymphoma flow cytometry, and improving coordination with labs and providers to obtain testing.

Case of Allergic Bronchopulmonary Aspergillosis in a Teenager with Crohn's Disease on Infliximab

Authors: Fain, Mary Ellen; Fitzpatrick, Nicolas; Goggin, Kathryn; and Vala, Snehal Vala

Presenting Author: Mary Ellen Fain, MD

Poster Number: 40

Introduction: Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity response to Aspergillus fumigatus typically seen in patients with asthma or cystic fibrosis. Increased Th2 CD4+ cell response to aspergillus leads to increased cytokines, eosinophilia and elevated IgE – hallmarks of the disease. We report a unique presentation of ABPA in a patient with Crohn's disease treated with infliximab.

Description: A 14-year-old female with Crohn's disease and allergies was referred to pulmonology for asthma evaluation. She reported one year of worsening cough, shortness of breath, and chest pain relieved by albuterol. Spirometry revealed moderate obstructive defect without bronchodilator response. Chest x-ray showed bilateral upper lobe pneumonia. She was admitted for workup and IV antibiotics. A non-contrast CT chest demonstrated bilateral posterior upper lobe consolidations with tree-in-bud nodularity, without cavitations or bronchiectasis. Bronchoscopy found tenacious secretions in bilateral upper lobes. Bronchoalveolar lavage cell count showed eosinophilia (16.7%), and culture was bacteria-negative, but identified aspergillus within 4 days. On CBC differential, absolute eosinophil count was 2,580 cells/microL. Further history revealed patient is an avid horseback rider and develops respiratory symptoms in the stable. A total serum IgE level was obtained and elevated (3,107 kU/L). An ABPA panel showed specific IgE to Aspergillus on immunoassay and precipitating IgG antibodies to Aspergillus. She was started on prednisone 0.5 mg/kg/day for one week, then every-other-day for 8 weeks, then a taper over 6 weeks. She was referred to infectious diseases who began 16 weeks of voriconazole for ABPA treatment and risk of progression to invasive pulmonary aspergillosis due to immunocompromise. The patient's symptoms improved, though some chest tightness and moderate obstructive defect on spirometry persist.

Discussion: We describe a unique presentation of ABPA in a teenager on infliximab for Crohn's disease. While ABPA may have been coincidental in this atopic patient, case reports in adults with other autoimmune diseases saw ABPA develop after initiating treatment with anti-TNF-alpha antibodies. It has been proposed that type-1 versus type-2 inflammation imbalance from Th1 inhibition may lead to relatively unopposed Th2 activity, and therefore increased likelihood of ABPA. To our knowledge, this is the first such case reported in the pediatric population.

Evaluating the Therapeutic Potential and Safety Profile of Tankyrase Inhibitors for HIV Cure

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Presenting Author: Riri Hamid, MS

Poster Number: 42

BACKGROUND: Despite the success of antiretroviral therapy (ART) at controlling viral replication, HIV persists in a reservoir of latently-infected memory CD4+ T-cells maintained through proliferation. While research efforts focus on reversing latency to promote infected cell clearance, achieving a cure for HIV for the 38.4 adults and 1.4 million children living with HIV will also likely require blocking reservoir cell proliferation. The Wnt/β-catenin signaling pathway that regulates T-cell proliferation and is implicated in maintaining HIV persistence thus represents an attractive target for HIV cure. Here, we explored a novel approach targeting a major regulator of Wnt/β-catenin pathway with Tankyrase inhibitors (TNKSi).

METHODS: We evaluated 6 TNKSi for their ability to (i) reverse HIV latency in the latently-infected J-Lat 10.6 expressing GFP upon HIV reactivation and in CD4+ T-cells from ART-suppressed SIV-infected rhesus macaques (RMs) and (ii) inhibit homeostatic proliferation ex vivo. Latency reversal was assessed by quantification of cell-associated HIV RNA levels by qRT-PCR and flow cytometric analysis of GFP expression. Inhibition of proliferation was determined using a cell division tracking dye in primary cells. To assess the safety profile of Tankyrase inhibition in vivo, 2 healthy RMs were treated with daily oral doses of Niraparib for 21 days. Longitudinal analyses included clinical evaluation, complete blood counts, serum chemistries, T-cell immunophenotyping and pathological review of bone marrow and rectal tissues.

RESULTS: Latency reversal was observed in 3/6 TNKSi tested with minimal cytotoxicity. Niraparib induced the greatest latency reversal as well as homeostatic T-cell proliferation inhibition and was thus selected for evaluation in nonhuman primates. Oral administration was well tolerated by the RMs with no clinical adverse events observed. However, we note an infiltration of immune cells in the rectal lamina propria of one RM treated with Niraparib. While minimal variations in T-cell subset frequencies were seen throughout the study, apoptosis was increased in memory CD4+ T-cells following Niraparib treatment in both RMs.

CONCLUSIONS: We identified Niraparib as a novel latency reversing and anti-proliferative agent with a good safety profile in nonhuman primates. Ongoing work is further investigating its immunomodulatory activities and will establish TNKSi therapeutic potential in pediatric HIV.

Decade-Long Hypogammaglobulinemia Following Autologous HSCT with Rituximab Containing Conditioning Regimen for Autoimmune Disorders: Clues from B and T Cell Profiling and Functional Studies

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Presenting Author: Sakshi Malik, PhD

Poster Number: 43

Background: Autologous hematopoietic stem cell transplantation (HSCT) utilizing rituximab and cyclophosphamide has been used in the management of refractory autoimmune disorders such as multiple sclerosis, systemic sclerosis, and other autoantibody-driven diseases. Rituximab (RTX) is effective in selectively depleting B cells and is generally considered safe. However, some patients may experience long-term hypogammaglobulinemia, requiring IVIG replacement. The mechanisms underlying persistent hypogammaglobulinemia remain poorly understood. In this report, we present immune profiling data from two unrelated female patients who developed persistent hypogammaglobulinemia more than a decade after receiving RTX and autologous HSCT for refractory systemic lupus erythematosus (SLE) and polymyositis.

Methods: Deep immunophenotyping was performed in one SLE and one polymyositis patient. Bulk RNA-seq of naïve-B cells and circulating T follicular helper cells (cTfh), as well as in vitro stimulation assay, were performed to assess immune dysregulation and naïve B cell maturation potential.

Results: We report that although both patients with persistent hypogammaglobulinemia recovered their B cell counts, they exhibited absence of class-switched memory B cells, while the frequencies of naïve B cells, transitional B cells, and double-negative B cells was comparable to that of healthy controls. cTfh cells were present at higher frequencies in both patients, with 60-70% skewed toward a $CXCR3^+$ Th1 phenotype, compared to 30-40% in controls. Bulk RNA sequencing of cTfh cells revealed an enrichment of genes associated with interferon responses, particularly IFN-γ. In vitro, B cells from patients exhibited intact class-switching when stimulated with IgM cross linking, CD40L and IL-21. However, when B cells were co-cultured with autologous cTfh cells, class switching was significantly reduced compared to controls. This suggests that impaired Tfh help from autologous cTfh cells, due to their biased $CXCR3^+$ phenotype, may contribute to the defective class-switching observed in these patients.

Conclusions : Our data show that the B-cell compartment in patients with persistent hypogammaglobulinemia contains quantitatively normal B cell numbers but exhibits an in-vivo B cell maturation defect. However, these B cells retain class-switching capability in-vitro when stimulated with T cell help factors. Additionally, Th1 skewed cTfh cells showed an increase in IFN-γ production upon stimulation. We propose that the higher frequency of $CXCR3^+$ cTfh cells may impair class-switching due to their limited helper function and altered migratory capacity.

The Impact of HIV-1 RT Mutations on Strand Displacement of the Central Polypurine Tract

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Presenting Author: Sri Dhanya Muppalla

Poster Number: 44

Human Immunodeficiency Virus (HIV) is a retrovirus that could lead to Acquired Immunodeficiency Syndrome (AIDS) without treatment (Swinkels et al., 2024). Reverse transcriptase (RT) is a key enzyme involved in the HIV replication cycle and is one of the main enzymes currently targeted by antiretroviral therapy. RT converts positive-sense single stranded RNA into a linear double stranded DNA form that integrates into the host genome (Herschhorn et al., 2010). During reverse transcription , strand displacement of the polypurine tracts (PPTs) is required to open the circular intermediate into the double-stranded DNA (Martin-Alonso et al, 2022). The Sarafianos lab recently solved the Cryo-EM structure of HIV-1 RT/strand displacement complex, which revealed several key interactive residues such as W24 and F61. To validate this mechanism, mutagenesis was performed on these residues and performed in-vitro urea-PAGE-based primer-extension assay. W24A and F61A mutants significantly reduced RT's strand displacement activity, validating that these residues are essential for strand displacement. This research deepens our understanding of HIV-1 RT's mechanism on strand displacement, potentially leading to the development of novel antiretrovirals.

Multidisciplinary Studies of HIV-1 Reverse Transcriptase Mutants with Nucleoside Analogs

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Presenting Author: Shreya Ravichandran, BS

Poster Number: 45

Background: 40 million people live with human immunodeficiency virus (HIV) (UNAIDS 2024), and 75% access antiretroviral therapy (ARTs) treatments. HIV reverse transcriptase (RT) is a common ART target, with multiple inhibitor classes. Two classes are nucleoside analogs binding the polymerase active site: nucleoside reverse transcriptase translocation inhibitors (NRTTIs) and nucleos(t)ide reverse transcriptase inhibitors (NRTIs). Islatravir (ISL; EFdA) is the first NRTTI, potently targeting RT; meanwhile, tenofovir (TFV) is an FDA-approved, routinely administered NRTI. Although both are efficacious, a common RT mutation is M184V, conferring low ISL resistance but TFV hypersusceptibility, exacerbated by the A114S mutation. As both inhibitors are adenosine analogs that bind the same site, it is perplexing how M184V/A114S RT has contrasting profiles to these compounds.

Methods: To determine structural features causing these resistance profiles, we use X-ray crystallography and cryogenic electron microscopy to solve structures of M184V and/or A114S RT, nucleic acid substrate, and each compound. To assess the mechanisms causing contrasting resistance profiles and to identify the compounds' IC50s against mutants, we use primer extension assays. Furthermore, we determine the EC50s of ISL and TFV within the TZM-GFP cell line subjected to single-round infection by virus-like particles (VLPs).

Results: We have solved four crystal structures: (1) M184V, (2) A114S, and (3) M184V/A114S RT with EFdA-TP and (4) M184V/A114S with TFV-DP, all with double-stranded DNA substrate. Additionally, we find deficiencies in substrate extension among mutants, visible in primer extension assays. Moreover – in agreement with previous studies of M184V/A114S RT – we observe less effective inhibition of M184V/A114S VLP infection upon ISL treatment and more potent inhibition by TFV treatment using our TZM-GFP reporter system.

Conclusion: In ongoing efforts, we report correlative changes in RT biochemistry and virus infection derived from the M184V/A114S mutations. Through structural studies, we see catalytic site changes from these mutations, potentially explaining decreased catalytic efficiency seen in biochemical studies; these differences are corroborated by primer extension assays. Our studies provide a multidisciplinary view to suggest compatible ART combinations as novel treatments.

Inactivation of Influenza A/B Using Methods Provided by Zeptometrix for Use on Antigen-Based Tests

Authors: Sabino, Courtney; Bowers, Heather; Sullivan, Julie; Lam, Wilbur; Schinazi, Raymond; Rao, Anuradha; and Bassit, Leda

Presenting Author: Courtney Sabino, BS

Poster Number: 47

Background: As a part of the RADx/ITAP program, Emory evaluates and determines the efficacy of SARS-CoV-2 / Flu A&B multiplex assays. In many Point-of-Care facilities where molecular tests are being used to diagnose patients, inactivated virus is the only option. Emory was prompted to determine just how much, if anything, different methods of inactivation affect Influenza A and Influenza B on antigen-based tests. Common inactivation methods were considered for evaluation, and it was determined that heat inactivation was an easily accessible option to many laboratories. Additionally, a commercially available option was also selected to be evaluated. Finally, the protein levels of each virus were determined.

Methods: Influenza A H3N2 obtained from BEI, and Influenza B, obtained from BEI was given to Zeptometrix so they can perform their proprietary inactivation methods. Once inactivated, the stocks were then sent back to Emory along with an aliquot of the still infectious live virus. Each virus was serially diluted so that a limit of detection could be determined. We obtained Ct values using our laboratory-determined tests (LDT), obtained ELISA data to determine pg/mL of protein levels, and tested them on an antigen-based LFA to determine any differences.

Results: While influenza A experienced little to no changes between live, heat inactivation, and PROtrol, influenza B was heavily affected by heat inactivation when it comes to LFA detection.

Conclusions: Heat inactivation severely affects Influenza B detectability when using an antigen based LFA. However, PROtrol remains a viable option for laboratorys looking to work with inactivated influenza B virus without losing any antigen sensitivity. Both heat inactivation and PROtrol can be used as an inactivated alternative to live influenza A when tested LFAs.

Hybridoma-based Generation of Monoclonal Antibodies Specific to Rhinovirus C2

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Presenting Author: Savannah Shooter, BS

Poster Number: 48

BACKGROUND: Rhinovirus (RV) is the leading cause of the common cold and a major trigger for asthma and COPD exacerbations. Despite its widespread impact, no approved antivirals or vaccines exist. Among the three RV species (A, B, and C), RV-C is particularly significant in children, with RV-C2 frequently detected in hospitalized cases. To advance research and therapeutic development, we generated monoclonal antibodies (mAb) targeting RV-C2. These mAbs could serve as valuable tools for investigating virus-host interactions and contribute to future diagnostic and therapeutic strategies.

METHODS: BALB/c mice were immunized intramuscularly five times using the TriVax method with an RV-C2 peptide encoding the 2A protease region of the VP1 protein, combined with poly(I:C) and anti-CD40 to enhance the immune response. Following the final immunization, splenocytes were harvested and fused with P3X63Ag8.653 cells to generate hybridomas. More than 600 hybridoma clones were obtained through HAT and HT selection, followed by limiting dilution. The specificity of the clones was confirmed by ELISA and Western blot analysis. Mouse serum collected after the final immunization served as a positive control.

RESULTS: Using hybridoma technology, we successfully generated mAbs specific to RV-C2. Over 90% of the hybridoma clones secreted IgG antibodies that recognized the RV-C2 peptide. However, only 23 of these clones exhibited strong IgG reactivity against the native RV-C2 virus. In the ELISA assay, clones were considered positive if their IgG titers were tenfold higher than those of the control clone. Of these, 18 clones demonstrated strong binding affinity to the native RV-C2 virus in Western blot analysis. We are currently evaluating whether these clones possess neutralizing activity against the native RV-C2 strain.

CONCLUSIONS: RV-C2 peptide-based immunization using the TriVax method efficiently induced a high frequency of peptide-specific mAbs. However, only a fraction of these mAbs exhibited reactivity to the native virus, highlighting the critical role of conformational epitope recognition. These results underscore the limitations of linear peptide immunogens in generating broadly reactive antibodies and the necessity of rigorous screening to identify clones that recognize native viral structures. Ongoing neutralization assays will determine the therapeutic and diagnostic potential of these RV-C2–specific antibodies.

Helping Young Students Build Healthy Habits Early That Will Last Their Lifetime Using AI-generated Video Lessons

Authors: Lee, Leigha; Abadiotakis, Helen; Williams, Tyler; Agyepong, Stephen; Myers, Kristin; and Pendley, Andrew

Presenting Author: Leigha Lee, BA, BS

Poster Number: 52

Introduction: Healthy lifestyle habits are an integral but often underemphasized part of a child's education, despite long-term impact on health outcomes. In Georgia, there is no standardized curriculum for healthcare education, leading to significant variability in delivery.

The Elementary Pediatric Health Curriculum (EPHC) addresses this gap through a longitudinal curriculum tailored for K-5 students. In partnership with Burgess Peterson Academy (BPA), an Atlanta elementary school (65% minority/31% economically-disadvantaged), EPHC delivers lessons over the academic year using its network of local healthcare-professionals/students.

EPHC seeks to expand this curriculum to students' families by creating accompanying take-home videos. These videos feature a cast of diverse characters designed to resonate with BPA families, transcending cultural/age barriers and ensuring critical health messages are reaching the broader Atlanta community.

Methods: EPHC has developed 11 lessons covering topics such as hydration and oral hygiene, delivered annually since 2018, and tailored to meet the developmental needs of students across grades K-5.

Recognizing the potential for greater scalability and inclusivity, EPHC is adapting these lessons into videos featuring fictional characters Robby and his mom, Dr. Robbowell. Using the AI-powered software Powtoon, our approach allows for rapid production while ensuring accessibility across literacy levels.

Initial production focuses on one pilot video, set to debut at BPA's PTA meeting in February 2025. Our group will seek feedback from parents/faculty to assess content relevance, cultural resonance, and perceived impact, before rolling out the first installment of 5-videos and optional survey to 100% of families in early spring, with Amazon gift card raffles to incentivize participation.

Results: Preliminary anecdotal evidence indicates students' ability to recall and apply these concepts across multiple grade levels, highlighting the curriculum's effectiveness.

The expansion to video format aims to amplify EPHC's impact on families, ensuring that habits can be reinforced outside of school. We anticipate preliminary engagement/adoption metrics to be available by the conference date.

Conclusions: By integrating innovative tools into health education, EPHC seeks to address systemic barriers to health literacy.

The Degree of Adiposity Enhances the Effect of the PNPLA3 rs738409 on Hepatic Steatosis in Prepubertal Latinx Children

Authors: Sanchez-Torres, Cristian; Ramirez Tovar, Ana; Gillespie, Scott; Huneault, Helaina; Knight-Scott, Jack; Alazraki, Adina; Khanna, Geetika; Yaranga, Claudia; Santoro, Nicola; Aguayo, Liliana; Bai, Shasha; Welsh, Jean; and Vos, Miriam B

Presenting Author: Ana Ramirez Tovar, MD (presenting for Cristian Sanchez-Torres, MD)

Poster Number: 54

PNPLA3 rs738409 is associated with hepatic steatosis (HS) and worst liver-related outcomes. In adults adiposity significantly amplifies the effect of PNPLA3 variants on HS; however, the synergism between PNPLA3 polymorphism and adiposity on HS in younger population needs to be tested.

Preliminary, secondary analysis of data from an ongoing clinical trial (NCT05292352) enrolling Latinx children aged 6-9 years. At baseline, liver fat quantification was completed using MRI-PDFF, and DNA was genotyped. All body measurements were performed twice by trained staff, and body mass index (BMI), BMI percentile (BMIp), and z-scores were calculated using pediatric CDC criteria. Univariable and multivariable logistic regression analyses were conducted to assess the association between PNPLA3 G-allele (CC vs. CG vs. GG) and BMI z-score on HS (defined as hepatic fat ≥5%) after controlling for covariates. Interaction between adiposity and genotype on HS was also tested.

At baseline, 98 out of 122 (80 %) enrolled children had PNPLA3 rs738409 genotype: 20% CC, 50% CG and 30% GG. Groups did not differ significantly by age or gender. The association of the GG polymorphism with HS increased with BMIp. Among children with BMIp less than the 85th, the median of HS between the three groups was less than 5% with higher values among carriers of the GG genotype compared with CC (2.80% vs. 1.50%). This difference was enhanced among children with a BMIp >95 where the median of HS of the GG carriers was more than double the percentage of the CC carriers (8.10% vs. 3.40%). After controlling for sex, age, and BMI z-score, PNPLA3 GG carriers were nearly 20 times more likely to have HS than CC (OR 19.93, p=0.003), and CG carriers nearly 6 times more likely than CC (OR 5.61, p=0.069). A multivariable model that included PNPLA3, BMI z-score, and ALT levels demonstrated an Area Under the Curve of 0.91. The interaction between PNPLA3 and BMI z-score did not reach statistical significance (p=0.13).

The association of the PNPLA3 rs738409 variants with risk of HS were stronger among children with obesity. Our findings suggest a potential need for patient oriented, precision intervention in genetically predisposed children. Analysis in larger and more racially and ethnically diverse population will be needed to confirm and understand the generalizability of these findings.

Determinants of Low Birth Weight among Singletons in the United States, 2014-2023

Authors: Burjak, Chloe; and Cordero, José

Presenting Author: Chloe Burjak, MS

Poster Number: 55

Background: Low birth weight (LBW) has significant etiologic heterogeneity and can arise due to gestational age or fetal growth patterns. The aim of this study is to examine the main determinants of low birth weight in the United States based on if the infant is born term or preterm and small, adequate, or large for gestational age.

Methods: We analyzed 37.8 million births from The National Vital Statistics System database to calculate descriptive data, unadjusted risk ratios, and time trends. We used 6.5 million births to construct log-binomial models to analyze how determinants of low birth weight differ based on prematurity, small for gestational age (SGA), and adequate or large for gestational age (AGA/LGA).

Results: LBW was associated with multiple factors, but they varied depending on preterm, term, SGA, and AGA/LGA. In preterm infants, biological factors like Non-Hispanic Black race [aRR=1.3, 95%CI(1.31, 1.32)], eclampsia [aRR=1.2, 95%CI(1.23, 1.28)], and chorioamnionitis [aRR=1.3, 95%CI(1.26, 1.30)] were significantly associated with LBW. However, socioeconomic and pre-pregnancy factors like having a previous preterm infant [aRR = 1.0, 95%CI(1.01, 1.03)] and maternal education [aRR = 1.0, 95%CI(1.00, 1.02)] were insignificant. In contrast to PTB, these factors were significant in SGA LBW infants.

Conclusions: The pathways to LBW are heterogeneous and vary by gestational age and fetal growth patterns. This suggests that distinct determinants may influence LBW across different birth pathways, such as preterm, term, or small, adequate, or large for gestational age, with varying factors at play for each pathway.

Establishing Developmentally Appropriate Benchmarks for Social Communication between Non-Autistic Peers

Authors: Bien, Elena; Edmier, Kathleen; Naresh, Aparna; Keleher, Courtney; Argueta, Tracy; Edwards, Laura; Yosick, Rachel; and Lampert, Erica

Presenting Author: Elena Bien, BS

Poster Number: 58

During peer interactions, preschool children with autism have shown lower rates of prosocial behaviors (initiations and responses) between non-autistic and autistic peers within an inclusive classroom. Interventions encouraging social communication are an important way to support developing meaningful social relationships, positive mental health, and later academic success in both autistic and non-autistic children. Existing assessment tools design intervention goals by comparing individuals against established criteria. These tools, however, do not provide specific benchmarks for social communication rates and frequencies at developmental age groups. This study will specify social communication benchmarks from non-autistic populations to help practitioners set developmentally appropriate intervention goals for children on the autism spectrum. Data was collected from 19 preschool children (2-5 years old) with four video recordings per child. This current work shares our preliminary coding analysis from three, 4-year-old individuals. Three 4-year-old, non-autistic, preschool children participated in a 10-minute free play session with a familiar teacher. Free play classroom videos were recorded and hand coded for social communication behaviors. Two trained coders independently recorded the frequency of verbal behavior for each child. Initial intercoder agreement was calculated and disagreements were discussed together until arriving at consensus. Social communication was operationalized as the frequency and variety of vocal verbal operants (VVOs) coded as mands (requests), tacts (labels), sequelics (two exchanges between a listener and speaker), and conversational units (three exchanges between a speaker and listener). During the 10-minute recorded interaction, the three, 4-year-old preschool students emitted an average of 26 total verbal operants (range 7-49 VVOs), 11 mands (range 8-17), 8 tacts (range 6-12), 2 sequelics (range 0-5), and 12 conversational units (range 1-20). This preliminary data begins to inform specific verbal operant benchmarks that are typical for four-year olds during free play with peers. More research is needed to further determine what developmentally appropriate goals may be for the frequency and variety of verbal operants in non-autistic preschool children. Future analysis will include video data coded for 19 preschool individuals.

Relationship Between Language Outcomes and Trajectories of White Matter Microstructure in Early Human Infancy

Authors: Driggers, Abigail; Walum, Hasse; Ford, Aiden; and Shultz, Sarah

Presenting Author: Abigail Driggers, BS *(not present)*

Poster Number: 59

Background: While young children become proficient in language comprehension and usage nearly universally, this phenomenon is nevertheless characterized by substantial variability with long-term developmental consequences. Language skills often emerge during the first two years of life, a period also characterized by rapid brain development. Much of the current literature aiming to characterize possible relationships between brain development and future language outcomes has focused on single-timepoint white matter tract maturity in newborns or infants older than six months, potentially obscuring individual differences in neurodevelopmental trajectories that could have meaningful impacts on language development. We hypothesize that - by investigating relationships between language outcomes and trajectories of white matter maturation in infants - we may establish candidate white matter tracts that may be investigated for their utility in predicting early language outcomes.

Methods: Typically developing infants (n=61, 36 male) completed diffusion tensor imaging (DTI) up to three times between zero- to six-months of age. Generalized additive mixed models were fit to visualize potential relationships between age-related changes in fractional anisotropy - an index of microstructural integrity in white matter - and language outcomes measured with the Mullen Scales of Early Learning (MSEL) at one or two years of age.

Results: Preliminary evidence was found for minor, transient differences in white matter trajectories differentially related to later domains of language development, including for left hemisphere pyramidal tract development in infants who went on to score highest in language measures around their first birthday.

Conclusions: These preliminary results suggest that relationships between trajectories of white matter maturation in the first six months and later language outcome should be further investigated. We have generated hypotheses regarding potential biomarkers for divergent language development, with early development of the left pyramidal motor tract as one candidate. Establishing the relationships of language outcomes with early development of white matter tracts such as these may help to inform the timing and targets of interventions to improve language development in vulnerable populations.

Anxiety-Related Differences in the Structure of Parent-Reported Autism Symptomatology through the Social Communication Questionnaire

Authors: Kolios, Alexander; Myers, Sarah; Kemp, Megan; Hann, McKenzie; Walum, Hasse; and Klaiman, Cheryl

Presenting Author: Alexander Kolios, BS

Poster Number: 61

Background: Symptomatology overlaps in anxiety and autism (ASD) make clinical distinctions challenging. Understanding the nature of these presentations and the components of ASD-evaluations most impacted by co-occurring anxiety symptoms is essential.

Methods: Clinician-evaluation analyses included 163 child-caregiver dyads, chosen based on completion of an Autism Diagnostic Observation Schedule (ADOS-2; child-assessment) Module 1, 2, or 3, and a Child Behavior Checklist (CBCL; parent-report). Caregiver-evaluation analyses included 6,262 dyads, chosen based on completion of a Social Communication Questionnaire (SCQ; parent-report) and a CBCL. All children had an ASD diagnosis and were 7 to 12 years old. All dyads were recruited through the SPARK cohort. An exploratory factor analysis (EFA) was administered on the SCQ questions. Linear regressions were performed between caregiver-reported (CR) anxiety scores and both ADOS total scores and SCQ factors. Mediation analyses were also used to investigate variables that may contribute to the relationships between anxiety and ASD.

Results: The EFA on the SCQ questions revealed three factors, two of which resemble the ADOS-2 Social Affect (SA) and ADOS-2 Restricted Repetitive Behaviors (RRB) categories. Anxiety symptom scores were found be a significant predictor of the "RRB" SCQ factor ($\beta = .235$, $p < .001$, $R^2 = .0551$). Higher anxiety scores also significantly predicted lower ADOS SA scores and RRB scores ($\beta = -.225$, $p = .004$, $R^2 = .0504$; $\beta = .231$, $p = .003$, $R^2 = .0532$). CR "Obsessive Compulsive Problems" (OCPs) scores were shown to significantly mediate the relationship between CR anxiety scores and "RRB" factor scores on the SCQ (ACME = .017, $p < .001$).

Conclusions: While these findings indicate that co-occurring anxiety symptoms may influence clinicians' assessment of children, their own ratings of anxiety did not factor into this relationship, meaning they didn't attribute these differences to anxiety. Caregivers' tendency to rate children with higher levels of anxiety as having higher RRBs may be connected to their ratings of OCPs, which could reflect difficulty in differentiating RRBs and OCPs. These findings highlight the differential perceived presentations of children with co-occurring anxiety symptoms and ASD as well as the need for increased insight into the role that anxiety plays in ASD diagnosis.

Cerebrospinal Fluid Stroke Volume is Decreased in Pediatric Chiari 1 Patients

Authors: Williams, Brice; Uribe, Bliss; and Oshinski, John

Presenting Author: Brice Williams, MS

Poster Number: 63

Background: Studies using Phase Contrast Magnetic Resonance (PCMR) have shown that peak cerebrospinal fluid (CSF) velocity is increased in patients with Chiari Malformation I (CM-I) compared to normal subjects, and that peak CSF velocity decreases after posterior fossa decompression surgery. However, peak velocity has not gained clinical acceptance as a diagnostic marker because noise, aliasing, and potential contamination of CSF measurements with vascular flow have hindered its applicability in practice. The volume of fluid that moves forward (or backward) through the foramen magnum per heartbeat – CSF stroke volume – is a more robust metric to assess CSF dynamics as it integrates velocity across the entire CSF cross-sectional area. We hypothesized that CSF stroke volume would be lower in pre-surgical CM-I patients than in control patients and would increase following surgery.

Methods: 49 pediatric CM-I patients (age 9.4 +/- 4.7) underwent MR imaging as part of normal clinical care. 15 patients underwent decompression surgery and 10 patients had follow-up MRI at 6 months. 8 additional volunteers (age 7.8 +/- 5.4), who underwent MRIs for non-specific headache symptoms, and were determined to be normal in structure and function by a neuroradiologist were used as control subjects. ECG-gated 2D phase-contrast magnetic resonance (PCMR) scans were acquired in the axial orientation at the foramen magnum (FM) and at C6 spinal vertebra. CSF stroke volume was calculated from flow by separately integrating positive and negative flow, then averaging.

Results: The stroke volumes in the pre-surgical CM-I subjects at FM were lower than the control subjects (n=46, p=0.028). Stroke volumes at FM were not significantly different in post-surgical CM-I subjects compared to controls (n=19, p=0.156). Pre-surgical stroke volumes were not different than unpaired post-surgical stroke volumes at FM or C6 (n=44). However, pre-surgical stroke volumes were lower than paired post-surgical at C6 (n=6, p=0.007). Additionally, paired pre-surgical volumes between FM and C6 were different (n=34, p=0.029) while paired post-surgical volumes were not different (n=8, p=0.394).

Conclusions: Pediatric control subjects had higher CSF stroke flow volume than CM-I patients. Sub-occipital decompression surgery increased CSF stroke volume in CM-I patients to values equal to normal control subjects. Paired pre- and post-surgical volumes showed significant differences while unpaired did not, suggesting each patient must serve as their own baseline for comparison. Further investigation is necessary to determine if patient demographics (age, height, weight) can serve to stratify stroke volumes.

Ethnoracial Disparities in Psychiatric Conditions, Criminal Legal Outcomes, and Case Processing among Youth in a Juvenile Court Diversion Program

Authors: Hines-Wilson, Mackenzie; Stielow, Sean; Sheerin, Kaitlin; Modrowski, Crosby; and Piper, Kaitlin

Presenting Author: Mackenzie Hines-Wilson, BA

Poster Number: 65

Background: Over 700,000 youth are arrested in the U.S. every year, and Black youth are 2.3 times more likely to be arrested and 71% more likely to be rearrested in comparison to their non-Hispanic White peers. However, unlike non-Hispanic White youth, ethnoracial minority youth are less likely to be offered opportunities to join diversion programs, which aim to redirect offenders from the justice system, and are more likely to receive harsher sentences for similar offenses. Less is known about juvenile racial/ethnic disparities within youth diversion programs. Therefore, this study explores disparities in behavioral health conditions, legal outcomes, and case processing of youth with mental health needs from a statewide court diversion program.

Methods: Data was collected using a mixed-methods approach via juvenile court record abstraction, surveys, and qualitative interviews with caregivers of youth with diversion program involvement (n=100). Most (58%) of the sample comprised ethnoracial minority youth.

Results: Bivariate analyses showed no significant differences in legal system outcomes (e.g., offense severity) or behavioral health scores between non-Hispanic White and ethnoracial minority youth in the diversion program. However, ethnoracial minority youth were significantly less likely to receive a mental health treatment mandate (24 [41%] vs. 26 [63%], p = 0.031). In the final logistic regression model, this difference was no longer significant after adjusting for behavioral health scores and legal outcomes. In qualitative interviews, families generally reported positive experiences with the diversion program. While they did not report discrimination within the program, many caregivers described experiencing bias in other legal settings (e.g., policing, incarceration).

Conclusion: Ethnoracial minority youth were less likely to receive a treatment mandate compared to non-Hispanic White youth. These disparities are primarily driven by behavioral health assessment results rather than race/ethnicity, suggesting the need for greater standardization in how assessments inform condition assignments, with consideration of how systemic inequities underscore the broader societal barriers faced by families of color involved in the legal system.

Preliminary Findings of Implementation of a Brief Mental Health Screener in a Pediatric Palliative Oncology Clinic

Authors: Radbill, Linda; and Largen, Kelsey

Presenting Authors: Linda-Maritza Radbill, PhD, and Kelsey Largen, PhD

Poster Number: 66

Background: Mental health screening is a standard of care for pediatric patients across a wide array of disciplines. However, no universal method for screening pediatric palliative care patients exists and information regarding mental health of palliative care patients at end of life is scarce. This study aimed to gather data from inpatient and outpatient palliative care patients to screen for mental health symptoms and to ensure patients in need of psychological services were appropriately identified.

Methods: From October 2024 to April 2025, we administered the PHQ-4 screening measure to pediatric patients followed by the outpatient or inpatient palliative care team. Patients screened at outpatient visits were administered a screener by nurses during their intake while patients admitted to the hospital were asked questions during psychology visits.

Results: During a 7-month time period, we collected a total of 65 screening measures for pediatric palliative care patients. Of these 48 were unique patient encounters. Patients ranged in age from 11-22 and the majority had an oncology diagnosis. Of the total screening, 53.8% of patients had little to no psychological distress; 35.4% had mild psychological distress; 6.2% had moderate psychological distress; and 4.6% had severe psychological distress. Almost all patients with moderate to severe psychological distress had been referred to psychology. Approximately half of those with mild psychological distress had been referred to psychology, and the majority of those who were not already had outside providers.

Conclusions: Mental health screening should be routinely integrated for pediatric palliative care patients and at regular intervals. Almost half of the patients had mild to severe psychological distress and for some patients this increased over time. As pediatric palliative care seeks to treat total pain in patients, assessment of psychological distress is critical in the provision of appropriate referrals and resources to provide holistic care.

Qualitative Barriers to Behavioral Health Treatment Among Families Involved in the Juvenile Legal System

Authors: Stielow, Sean; Hines-Wilson, Mackenzie; Piper, Kaitlin; Sheerin, Kaitlin; Modrowski, Crosby; and Kemp, Kathleen

Presenting Author: Sean Stielow, MS, MAT

Poster Number: 67

Background: Between 50-70% of youth involved in the juvenile legal system meet criteria for at least one psychiatric condition, compared to about 20% in the general adolescent population. To address this mental health crisis, juvenile diversion programs often screen youth for behavioral health conditions and refer them to providers. However, only a small proportion of youth in need of treatment initiate care during or after their involvement with the system (<10%). This study is to identify barriers to treatment participation among youth in a court diversion program with identified behavioral health needs.

Methods: Data collection involved surveys, administrative court records, and interviews with 100 caregivers and youth in a juvenile court diversion program in a northeastern state (July 2023-May 2024). Procedures were approved by the health system-affiliated IRB.

Results: Barriers identified by caregivers were coded into three domains: Obstacles and Stressors, Treatment Demands and Issues, and Perceived Treatment (Ir)relevance. Within the Obstacles and Stressors domain, caregivers were most concerned about challenges related to scheduling, long wait times between screening and treatment, and the difficulty of finding providers with available services. Within the Demands and Issues domain, caregivers voiced concerns about the cost of treatment, confidentiality when receiving services in a court setting, youth resistance to treatment, and cultural and identity-based compatibility between the provider and youth. Within the Treatment Irrelevance domain, caregivers expressed a strong interest in mental health services for their children but showed less enthusiasm for substance use treatment. Participants had varied perspectives on the need for treatment and services for caregivers of court-involved youth.

Conclusions: Caregivers expressed strong support for behavioral health treatment and overwhelmingly favored community-based services over court-affiliated options for their children. These findings underscore the importance of juvenile court diversion programs adopting a family-centered approach in their engagement with both caregivers and youth.

Provider-Led Discussion of Caregiver Adverse Childhood Experiences (ACEs) and Emerging Themes in Visits to Pediatric Acute Care Facilities

Authors: Trudeau, Rylea; Agarwal, Maneesha; and Lott, Abigail

Presenting Author: Rylea Trudeau, BS

Poster Number: 68

Background: Adverse Childhood Experiences (ACEs) are potentially traumatic experiences in childhood that are linked to a variety of negative health outcomes. Higher levels of ACEs in caregivers are associated with elevated ACE scores and diminished health outcomes among children, a higher frequency of pediatric ED visits, and inversely associated with higher acuity visits. Less is known about how screening and discussion of parental ACE-related impacts on parenting by pediatric healthcare providers may reduce child health risk or healthcare utilization.

Methods: The sample (N=94) comes from an ongoing longitudinal randomized-controlled trial of families enrolled during their child's routine well-child check appointments. Caregivers were given a survey about ACEs, then randomized into usual care or intervention groups (i.e., provider-led discussion about caregiver ACEs). Patient chart data on number of ED/urgent care visits with timestamps and diagnostic codes were recorded. As modeled by previous reports, diagnostic codes were standardized by body system categories. Patient visits were categorized by weekday vs weekend arrivals. Descriptive statistics were used to examine frequencies of these factors by group (n=69 usual care, n=25 intervention).

Results: Preliminary results show a total of 165 visits (mean = 2.39 per child) in the usual care group and 77 (mean = 3.08 per child) in the intervention group. Diagnostic codes assigned to each visit suggest the infectious disease category was most prevalent in the usual care and intervention groups (72.73% in both groups). Body system categories of endocrinology and mental/behavioral health were the least represented in both groups (0%). Both groups exhibited higher utilization on weekdays (72.12% in usual care, 83.12% in intervention) compared to weekends.

Conclusions: Preliminary results suggest higher utilization of pediatric emergency care services among the intervention group, with similar trends in diagnostic codes as categorized by body system. Continued identification and assessment of themes in presentation to pediatric emergency care facilities can offer insight into how discussing parental ACEs in a primary care setting influences pediatric healthcare utilization behaviors. Ultimately, these findings may help us better understand and support patients by providing responsive, trauma-informed care in a way that is of highest value to families.

Differentiating the Effects of Household Income and Maternal Education on Caregiver Interactive Behavior

Authors: Ravichander, Aanya; Shultz, Sarah; and Ford, Aiden

Presenting Author: Aanya Ravichander

Poster Number: 69

Background: Dyadic interactions between infants and caregivers are an early platform for brain and behavior development. Caregivers adjust their interactive behaviors during interactions to reflect their perceived understanding of their infant's social ability, and prior research suggests that socioeconomic status may affect this bidirectional, mutually adapted learning process. Few studies, however, have investigated whether different socioeconomic factors, like household income and maternal education, have separable effects.

The goal of this study was to delineate how household income versus maternal education predict developmental change in caregiver greeting – a distinctive, infant-directed signaling behavior – in dyads with neurotypically developing infants.

Methods: Infants and their caregivers (n=78) completed longitudinal recordings of live, screen-mediated interaction at up to six timepoints from 0-6 months of age. The presence of the greeting behavior (simultaneous widening of the eyes, eyebrows, and mouth) was manually coded at the beginning of each interaction. Socioeconomic measures of household income and maternal education (binarized to graduate degree or not) were obtained upon study enrollment.

Generalized Additive Models (GAMs) were used to evaluate the predictive effects of maternal education and household income on the use of greeting behavior from 0-6 months. A mediation analysis was used to evaluate if the effects of maternal education were explained by the effects of household income.

Results: Evaluation of model fit (using summary statistics and MSE) indicates that the likelihood of greeting is best predicted by incorporating both household income and maternal education as fixed effects. Both were significant predictors in the final model (income: $z=2.78$, $p=0.005$, education: $z=2.27$, $p=0.015$). The likelihood of greeting was higher in families without graduate degrees and with greater incomes. The mediation analysis showed that maternal education predicted greeting likelihood separately from household income ($p=0.022$).

Conclusion: Multiple socioeconomic factors predict age-related changes in how caregivers engage their infants during early interaction. While maternal education and household income are associated with each other, maternal education predicts greeting likelihood outside of household income. Continued studies investigating socioeconomic factors should incorporate multiple measures to more accurately capture the effects of socioeconomic status on infant learning and development.

Understanding the Impact of War and Trauma on Children. Is There an Emerging Global Health Crisis?

Authors: Shah, Zaria; Coulter, Colleen; Adamkiewicz, Tom; and Gill, Corey

Presenting Author: Zaria Shah

Poster Number: 70

Background: According to the UN, in 2023 5,301 children were killed and 6,348 were maimed – a 35% increase from 2022. 63% of explosive weapon casualties are civilians (AOAV, 2024), 20% to > 40% are children. In 2017, over 90,000 children (0-14 years) suffered unilateral lower limb amputations due to conflict and terrorism – the 3rd leading cause globally. Children are innocent victims. Although the news reports pediatric amputations during conflicts, data is limited in peer-reviewed literature.

Methods: To better understand optimal medical, surgical, rehabilitation, short- and long-term outcomes, utilizing PRISMA guidelines, the authors conducted a review of the literature on pediatric limb injuries in armed conflicts. Articles were extracted from academic libraries/Medline/Google Scholar and references identified by Jain RP et al. Key words searched included but not limited to "child, pediatric, amputation (severity and levels), global, trauma, war, conflict, surgical procedures, and rehabilitation." Additionally, a word cloud analysis was conducted on both groups of articles identifying frequency of words present in titles, abstracts, method, and result sections.

Results: Initially, 75 articles were identified, 62 peer reviewed, and 57 included limb injury/amputation in children during armed conflicts. 34 articles included data on children, with 27 articles including aggregated data on children and adults. Articles included similar frequent words (amputation, trauma, injuries, wound, patients). However, both groups of articles also differed, with the disaggregated reporting children, age, mortality, and location. The aggregated data group had more frequent mention of fracture, graft and nerve injury. This analysis indicates that both groups of articles provide different information requiring consideration. Barriers of care identified include access to appropriate medical care, long-term follow-up and care, and unreported data. Additional barriers identified include comorbidities; polytrauma support; complications, type of care facility (military, civilian, NGO), child specific resources medical supplies & equipment, high costs in conflict zones, lack of ortho/multidisciplinary expertise, prosthetic and rehab services, psycho-social family/community support, and systematic barriers (no trauma/limb loss registries, no/limited prevention strategies).

Conclusion: The purpose of the proposed systematic review is to identify the need for global resources and to increase awareness of the impact of conflicts on children.

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