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Mitochondrial metabolic flexibility is critical for CD8 T cell anti-tumor immunity

Authors: Chen, Chao; Araki, Koichi; Zhen, Hong; Zhao, Peng; Ford, Mandy L.; Ahmed, Rafi; and Qu, Cheng-Kui

Presenting Author: Chao Chen, PhD

Mitochondria are flexible in utilizing competing substrates for energy metabolism in accordance with the availability of nutrients and energy/biosynthesis demands. CD8 T cells in different functional states during the life cycle have distinct metabolic requirements, and they experience dynamic changes in energy sources and oxygen levels in various microenvironments. The significance of mitochondrial metabolic flexibility for CD8 cell immune responses has not been characterized. Here we report that the deletion or pharmacological inhibition of PTPMT1, a mitochondria-based multi-functional phosphatase, significantly decreased the expansion and effector specification of activated CD8 cells. The deletion of PTPMT1 from T cells accelerated tumor growth and impaired stem-like CD8 T cell development. PTPMT1 depletion also suppressed anti-viral CD8 T cell responses. Mechanistically, the loss of PTPMT1 critically altered mitochondrial fuel selection - the utilization of pyruvate, a major mitochondrial substrate derived from glucose (the simple carbohydrate), was inhibited whereas fatty acid and glutamate utilizations were enhanced. Persistent mitochondrial substrate shift and metabolic inflexibility induced oxidative stress and apoptosis in PTPMT1 knockout CD8 cells. The impact of the biased mitochondrial fuel selection on CD8 T cells was further verified in mitochondrial pyruvate carrier (MPC) knockout and pharmacological inhibition models. Taken together, this study suggests an important role of PTPMT1 in maintaining mitochondrial metabolic flexibility and that temporally and spatially regulated carbohydrate oxidation is essential for CD8 cell anti-tumor immunity
Early ART plus immune interventions to limit SIV reservoir establishment in infant rhesus macaques

Authors: Farinre, Omotayo; Anaya, Tzoalli; King, Lexy; Ehnert, Stephanie; Jean, Sherrie; Laird, Greg; Mason, Rosemarie; Roederer, Mario; Safrit, Jeffrey; Mavigner, Maud; and Chahroudi, Ann

Presenting Author: Omotayo Farinre, PhD

Background: Antiretroviral therapy (ART) successfully controls viremia in HIV-1 infection. However, persistent reservoirs of latently infected CD4+ T-cells remain the major obstacle to cure. In this infant nonhuman primate (NHP) model, we tested the hypothesis that a combination of interventions given at the time of ART initiation may restrict reservoir size by accelerating the loss of infected CD4+ T-cells. The interventions used included N-803, an IL-15 super-agonist known to enhance immune responses through activation and proliferation of T and NK cells, plus a cocktail of anti-SIV Env-specific rhesus IgG1 monoclonal antibodies (RhmAbs) to promote infected cell clearance through antibody-dependent cellular cytotoxicity.

Methods: Twenty-two infant rhesus macaques were orally infected with SIVmac251 at 4 weeks of life and started on ART +/- additional interventions 1-2 weeks post infection, divided into 3 groups: i) ART only, ii) ART+SIV RhmAbs, and iii) ART+SIV RhmAbs+N-803. Animals were longitudinally monitored for plasma viral loads and CD4+ T-cells were isolated from blood and lymph nodes and to measure the impact of the interventions on the viral reservoir using qPCR to quantify total SIV DNA/RNA and intact proviral DNA.

Results: ART suppressed viral loads below the limit of detection and no significant differences were detected in the decay of viremia across groups. Levels of CD4+ T-cell-associated DNA and RNA declined on ART in all groups with the greatest fold change between week 0-26 of ART found in the group that received SIV RhmAbs without N-803 (p=0.018 vs controls). However, intact proviral SIV-DNA did not differ between groups at week 26 in CD4+ T-cells from blood or LN. The group that received SIV RhmAbs + N-803 showed an unexpected increase in SIV-RNA at week 16 of ART in CD4+ T-cells from blood and LN, possibly as a delayed consequence of N-803-mediated immune stimulation.

Conclusions: Early intervention with ART in the infant rhesus macaques was effective in suppressing viremia. The addition of SIV RhmAbs to ART improved the elimination of infected cells but did not impact the level of persistent, intact SIV once viral loads were suppressed. Disproving our initial hypothesis, N-803 did not help to limit reservoir establishment.
Dual GD2 and PTK7 Targeted γδ T cell Immunotherapy for Neuroblastoma

Authors: Jonus, Hunter; Lee, Jasmine; Silva, Jordan; Shim, Jenny; Spencer, Trent; and Goldsmith, Kelly

Presenting Author: Hunter Jonus, PhD

Background: Adoptive cell therapy (ACT) for neuroblastoma (NB) has primarily utilized autologous products of alpha beta (αβ) T cells. Gamma delta (γδ) T cells offer a potentially superior and non-alloreactive alternative with innate NB cytotoxicity. We demonstrated preclinical potency of unengineered allogeneic γδ T cells in combination with anti-GD2 antibody and chemotherapy against NB, supporting a first-in-human clinical trial (NCT05400603). In our second generation γδ T cell therapy, we propose to introduce chimeric antigen receptor (CAR) expression to enhance efficacy. While GD2-targeted ACTs are well-tolerated, GD2 may downregulate following relapse leaving a need for novel NB-specific immunotherapy targets. We therefore identified protein tyrosine kinase 7 (PTK7) to be robustly expressed on the NB cell surface with minimal normal tissue expression using a novel pediatric normal tissue microarray.

Objective: Our primary objective is to bioengineer γδ T cells with dual CAR expression targeting both PTK7 and GD2 to improve tumor trafficking, minimize toxicity, and circumvent potential therapy resistance due to antigen escape.

Methods: Transgenes containing either GD2 or PTK7 scFv targeting domain, CD8 hinge, CD28 co-stimulatory domain, and CD3ζ signaling domain were designed for mRNA transfer to γδ T cells via co-electroporation. CAR expression was determined using flow cytometry and anti-NB cytotoxicity by annexinV-7AAD apoptosis assay.

Results: Simultaneous expression of both CARs appears highest 24 hours following electroporation, with ~70% of cells dually modified. Compatible with the in vivo lifespan of allo γδ T cells, CAR expression can be detected up to 72 hours after mRNA introduction. Anti-GD2/PTK7 γδ T cells are potent against IMR5 (GD2+PTK7+) with ~50% NB death at a 1:1 effector:target ratio. Dual-targeted γδ T cells show antigen-specific activity against GD2+PTK7+, GD2+PTK7-, and GD2-PTK7+, but not GD2-PTK7- NB models, engineered to represent potential clinical heterogeneity of target expression.

Conclusions: Dual PTK7/GD2 CAR expression in γδ T cells is feasible and yields strong anti-NB potency. Multiple infusions of the allogeneic donor-derived therapy can counteract a finite γδ T cell lifespan. Ongoing efforts include optimizing CAR signaling domains for maximal efficacy and confirming efficacy in vivo with the ultimate goal of rapid clinical translation.
Pupillary Light Response Deficits In 4-Week-Old Piglets And Adolescent Children After Low-Velocity Head Rotations And Sports-Related Concussions

Authors: Oeur, Anna; Mull, Mackenzie; Riccobono, Giancarlo; Arbogast, Kristy; Ciuffreda, Kenneth; Joshi, Nabin; Fedonni, Daniele; Master, Christina and Margulies, Susan

Presenting Author: Anna Oeur, PhD

Background: Visual deficits are commonly reported after a traumatic brain injury (TBI). The pupillary light reflex (PLR) is an emerging diagnostic tool for concussion. The 4-week-old piglet is an established model of pediatric brain injury with similar neuroanatomical distributions and brain development stages as children. The aim of this study was to establish quantifiable PLR biomarkers of mild TBI in 4-week-old piglets using a clinically available handheld pupillometer. In parallel, we examined human PLR metrics, using the same assessment tool, in both healthy adolescents and those within 28 days of injury from a sports-related concussion (SRC).

Methods: An automated handheld PLR device (PLRTM-3000, Neuroptics Inc.) was used to obtain PLR in 4 week-old piglets allocated to a healthy group and an experimental group that experienced rapid nonimpact head rotation (RNR). The 95% PLR reference ranges (RR) for maximum and minimum pupil diameter, latency, and average and peak constriction velocities were established in healthy piglets (N = 13), and validated using nine additional healthy piglets. PLR assessments were obtained in piglets allocated to anesthetized sham (N = 10), single (sRNR, N = 13), and repeated (rRNR, N = 14) sagittal low-velocity RNR at pre-injury, and days 1, 4, and 7 post. Reference ranges were similarly established in healthy adolescents (N = 167) and compared to adolescent PLR within 28 days from SRC (N = 177).

Results: In piglets, maximum and minimum diameter deficits were greater in rRNR than sRNR. Alterations peaked on day 1 post sRNR and rRNR, and remained altered at day 4 and 7. In addition, piglet maximum and minimum diameters were significantly below the RR for rRNR compared to shams and their own baselines at day 1. In SRC adolescents, the proportion of adolescents within the RR was significantly lower for maximum pupil diameter only (85.8%).

Conclusions: We show that PLR deficits may persist in humans and piglets after low-velocity head rotations. Maximum pupil diameter is a metric sensitive to head rotations across species. We conclude that PLR is a feasible, quantifiable involuntary physiological metric of neurological dysfunction in pigs, as well as humans.
Single-cell profiling of acute myeloid leukemia identified ARMH1, a novel protein, associated with proliferation, migration, and drug resistance.

Authors: Bakhtiari, Mojtaba; Bhasin, Swati S.; Thomas, Beena E.; Mumme, Hope; and Bhasin, Manoj

Presenting Author: Mojtaba Bakhtiari, MD

Despite advancements in treatments, half of the pediatric acute myeloid leukemia (AML) patients relapse (Chen, J. & Glasser, C.L., 2020), making it pertinent to identify novel biomarkers that can be used for better stratification of patients post-end of induction (EOI) therapy as well as to develop targeted therapies that could lead to higher clinical remission (CR) rates. Single-cell RNA sequencing (scRNAseq) analysis has revolutionized cancer research by identifying novel cell types, genes, and pathways. Our group used scRNAseq analysis of pediatric AML bone marrow (BM) samples collected at diagnosis (Dx) and EOI to identify the upregulation of multiple genes including a novel gene, Armadillo-like helical domain containing 1 (ARMH1)/C1orf228, in cancerous-blast cells (Thomas, B.E. et al., 2020). ARMH1 has not been previously associated with AML. Also, ARMH1 expression was observed to be significantly higher at Dx in samples from patients with relapse compared to samples from patients with CR (P90%) compared to EOI samples (FC=1.8, P=.003) (Ulukaya, G. et al., 2021). Further analysis on other leukemias like T cell-acute lymphocytic leukemia (T-ALL) and mixed phenotypic acute leukemia (MPAL) also revealed higher expression of ARMH1 in the blast cells as compared to non-blast cells. Survival analysis on bulk RNAseq data using the survival genie platform (Dwivedi, B. et al., 2022) revealed that higher expression of ARMH1 was significantly associated with poor overall survival (OS) in both AML (P=.003) (Ulukaya, G. et al., 2021) and T-ALL (P=.014) datasets. Comparative gene expression analysis using the Pediatric Single Cell Cancer Atlas - PedSCAtlas - tool (Mumme, H.L. et al., 2021), revealed negligible expression of ARMH1 in normal lymphoid, myeloid, and erythroid cell lineages, further confirming the leukemic blasts-specific over-expression of ARMH1.

ARMH1 encodes a cytosolic protein of yet unknown function. The malignant blast specificity of ARMH1 suggested a role in leukemic cells' survival/maintenance. To ascertain its functional involvement in cancer cells, ARMH1 was knockdown using Lentivirus in both normal and AML cell lines. Through in vitro assays, we demonstrate that ARMH1 knockdown significantly reduced leukemic cells' capacity to proliferate in the shARMH1 in the HEL cell line (P=0.0012), Molm14 (<0.0001) and Kasumi-1 (<0.0001) and shARMH1 migration in HEL (P=0.0132), Molm14 (P=0.0005) and Kasumi-1 (P=0.0167). In addition, overexpression of ARMH1 with packaging ORF Lentivirus, we observed that cell proliferation increased, in HEL (P=0.0005). Moreover, cell migration is also increased in HEL (P=0.0001) and Molm14 (P=.0079).

We also observed slightly increased sensitivity to the chemotherapy drug, Cytarabine in the ARMH1 Knockdown cancer cells compared to control cancer cells in which the gene is fully functional. However, suppression of ARMH1 expression had no effect on cell growth in the normal HEK293T cell line (P=0.913).

In conclusion, we demonstrate for the first time that ARMH1, a still poorly understood protein, contributes to the increased proliferation and migration of malignant AML cells. With specific overexpression in blast cells of several pediatric leukemias and the newly discovered roles in proliferation, migration, and chemotherapy resistance, ARMH1 may be a good candidate for targeted treatment of pediatric leukemias.
Butyrate supplementation during pregnancy reduces injury in murine model of neonatal intestinal inflammation

Authors: Barbian, Maria E.; Naudin, Crystal; Denning, Patricia; Patel, Ravi M.; and Jones, Rheinallt

Presenting Author: Maria Barbian, MD

Background: The diet during pregnancy (antenatal diet) impacts fetal gut development. Butyrate is a short-chain fatty acid (SCFA), generated by gut bacteria, which improves intestinal health through dampening intestinal inflammation and enhancing the intestinal barrier. Previously, we demonstrated that antenatal butyrate supplementation (ABS) in mice reduces intestinal injury in adult offspring with experimentally-induced colitis.

Objective: Here, we evaluated whether ABS reduces intestinal injury in a murine model of neonatal intestinal inflammation. We hypothesize that ABS will reduce intestinal injury in neonatal offspring exposed to a model of intestinal inflammation via down regulation of inflammation and enhancement of the intestinal barrier through upregulation of tight junction proteins.

Methods: Mating pairs of C57BL/6 mice were assigned to an experimental or a control group. The experimental group received 1% butyrate in their drinking water throughout their pregnancy. Offspring underwent a model of intestinal inflammation or evaluation of in vivo intestinal permeability at 7, 10 and 14 days of age through oral gavage of fluorescein isothiocyanate-dextran 4 kilodalton (FD4). The 2-week-old offspring which underwent intestinal inflammation received an intraperitoneal injection of lipopolysaccharide and platelet activating factor. Intestinal injury was measured in blinded fashion through histopathologic scoring. Samples of ileum were obtained for gene enrichment analysis.

Results: The histopathologic analysis revealed offspring from the ABS group have lower injury scores than control offspring (p-value: 0.02). Through RT-qPCR from ileum tissue samples, offspring from the ABS group had 3.5-times higher levels of TGF-beta 1 mRNA expression (p-value: 0.02) and 90-times lower levels of NF-kappa B expression (p-value: 0.04). mRNA expression of tight junction proteins was similar between the two groups. Our preliminary findings from the intestinal permeability analysis suggests ABS does not alter the offspring’s intestinal permeability.

Conclusions: ABS resulted in reduced intestinal injury in neonatal murine offspring. This may be secondary to decreased inflammatory tone in the intestine of the offspring exposed to ABS as increased TGF-β1 and decreased NF-κB signaling dampen inflammation. From our preliminary findings, ABS does not appear to influence the neonatal intestinal barrier. Our results highlight the powerful influence of the diet during pregnancy on gut development and future gut health.
Evaluation of the Immune Checkpoint Siglec-15 in Promoting Immune Dysregulation in Pediatric Lymphomas

Authors: Francis, Dailia B.; Dougan, Jodi; Pillsbury, Claire E.; Park, Sunita; Liu, Linda N.; Porter, Christopher C.

Presenting Author: Dailia Francis, MD, PhD

Background: Non-Hodgkin’s lymphomas (NHL) are a heterogenous group of hematologic malignancies. Intensive multiagent chemotherapy regimens has dramatically altered the treatment landscape, improving the 5-year event free survival for this cohort of patients. However, outcomes in the relapsed and refractory setting remain dismal with survival <30%. As such, there remains an urgent unmet need for new and effective therapies in this population. Siglec-15 (Sig-15), an I-type lectin, is a critical immune suppressor that is highly expressed in various human cancers and intra-tumoral myeloid cells. Importantly, inhibiting Sig-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 Sig-15 in promoting disease progression in hematologic malignancies has not yet been described.

Methods: Sig-15 expression was evaluated in primary lymphoma patient samples as well as various lymphoma cell lines using western blot (WB), quantitative PCR as well immunohistochemistry (IHC) and immunofluorescence methods. The impact of NF-kB signaling on Sig-15 expression was interrogated in human lymphoma cell lines using various pharmacologic agents including phorbol myristate acetate (PMA), ionomycin, CD40-Ligand and BOT-46 which either induce or inhibit NF-kB activity. Sig-15 expression was then assessed by WB and PCR analysis. Sig-15 expression was inhibited through genetic downregulation in the well-established murine lymphoma cell line A20 and injected into immune competent and immune deficient mice to determine the effect on tumor progression and survival.

Results and Conclusions: WB shows higher Sig-15 expression in lymphoma cell lines compared to healthy donor cells. IHC of a tumor microarray and validation samples from children shows high Sig-15 expression in NHL samples with distinct staining patterns based on subtype. Stimulation of NF-kB signaling induces increased expression of Sig-15 in lymphoma cells and appears to stabilize Sig-15 in the presence of concurrent inhibition. Lastly, knockdown of Sig-15 in A20 cells abrogates disease progression in immune competent but not immunodeficient recipients, consistent with a role for Sig-15 in immune evasion in lymphoma. Together, these data implicate Sig-15 as an immune checkpoint that may be inhibited therapeutically to promote an immune response to lymphoma cells.
Time-Sensitive Changes in Serum Biomarkers following Rotational Head Injury in a Piglet Model

Authors: Huber, Colin; Thakore, Akshara; Oeur, Anna; and Margulies, Susan

Presenting Author: Colin Huber, PhD

Background: Traumatic brain injury (TBI) is common in sports and automobile accidents leading to neurophysiological deficits, and children aged 0-4 years are particularly vulnerable with high rates of TBI. Currently, TBI is diagnosed through clinical interpretation of symptoms and brain scans of hemorrhagic events; however, there is limited understanding of noninvasive, objective biomarkers to inform diagnosis and treatment. This study aimed to examine the time-course of glial fibrillary acidic protein (GFAP) and neurofilament light (Nf-L), biomarkers of glial and axonal injury, in serum (Simoa Human Neurology 4-Plex A assay) after TBI.

Methods: A retrospective analysis was conducted of serum samples collected from 4 week-old female piglets, representing children aged 2-3 years. Serum samples were collected at multiple timepoints pre-injury and up to 1 week post-injury following a sham or single sagittal rapid non-impact head rotation (RNR) injury (n=214). Changes in serum concentrations were evaluated using two-way ANOVA with interaction between treatment and timepoints. Post-hoc tests were conducted using one-way ANOVA across timepoints and two-sample t-tests between sham and RNR animals.

Results: Mean pre-injury levels of GFAP and Nf-L were 27.2 ± 18.0 and 28.2 ± 14.8 pg/mL with a healthy reference range (RR) established from the 2.5–97.5 percentiles: 7.3–66.8 and 10.9–65.8 pg/mL, respectively. Healthy RRs were validated using separate pre-injury samples, where 93% (GFAP) and 97% (Nf-L) fell within these RRs. Immediately after RNR, GFAP was significantly above pre-injury (at 30 minutes, 1 hour, and 6 hours post-injury, all p<0.067). In contrast, Nf-L increased after a brief delay, and remained elevated for RNR at 6 hours, 1 day, and 1 week post-injury compared to pre-injury (p<0.001) and also compared to sham at 1 week (p<0.001).

Conclusions: GFAP and Nf-L increased following a rotational head injury at two distinct time courses, showing promise as complimentary, objective measurements to inform early diagnosis and treatment of TBI.
Utilizing chemotherapy to enhance the anti-tumor properties of ex vivo expanded γδ T cells in acute myeloid leukemia

Authors: Jhita, Navdeep; White, Kinnede R; and Raikar, Sunil S

Presenting Author: Navdeep Jhita, M.D. B.S

Background: Pediatric acute myeloid leukemia (AML), has high rates of treatment failure and aggressive relapse. While allogeneic HSCT offers a cure, limitations arise in achieving remission prior to transplant. Of significance, immunotherapy-based approaches can induce remission. We explore gamma delta (γδ) T cells as a promising therapeutic tool. Allogeneic γδ T cells target in an MHC independent manner, thus have minimal risk of causing GvHD and creating a cellular therapeutic ideal to target AML. Cytotoxic mechanisms of γδ T cells include recognition of cellular stress molecules such as NKG2D and DNAM-1 receptor ligands on target cells, activation of death receptor pathways through Fas-FasL and TRAIL-R interactions, and γδ TCR mediated killing through butyrophilins (BTN) on target cells. Stress molecules are expressed on AML and can be upregulated through chemotherapeutic agents such as azacitidine and venetoclax, drugs currently used to treat relapsed AML. We plan to test these drugs in combination with γδ T cells to target AML.

Methods: Five leukemic cell lines (Kasumi-1, Nomo-1, MV4-11, MOLM13, CMK), were respectively treated at their corresponding inhibitory concentration 25 and 50 of azacitidine and/or venetoclax for 24 hours. After treatment, cells were assessed for stress molecules expression, elucidating which pathways aide in γδ T cell targeting and cytotoxicity. We co-cultured pre-treated AML cells with γδ T cells and measured cytotoxicity using flow-cytometry and a bioluminescence-based assay.

Results: Each cell line expressed a unique pattern of markers at baseline. Cell lines treated with azacitidine had a robust expression of ULBP1, ULBP2/5/6 and ULBP3. Azacitidine increased CD155 and TRAIL-R2 in Nomo-1 while CD112 increased in MOLM13. Most venetoclax-treated AML cells over-expressed BTN2A and BTN3A1 along with CD95 in MOLM13. Initial in vitro cytotoxicity studies indicate increased total cell death across multiple AML cell lines when γδ T cells were co-cultured with cells pretreated with azacitidine/venetoclax.

Conclusions: Azacitidine and venetoclax treatment resulted in upregulation of several markers involved in γδ T cell-mediated cytotoxicity. Ex vivo expanded γδ T cells combined with chemotherapy-treated AML cells resulted in increased overall cytotoxicity, which is likely both additive and synergistic. Our next steps include preclinical validation of this combinatorial approach in an in vivo mouse study.
Increased Level of IGFBP2 promotes Tumor metastasis in SHH Medulloblastoma

Authors: Kunhiraman, Haritha; McSwain, Leon; Shahab, Shubin; and Kenney, Anna

Presenting Author: Haritha Kunhiraman, PhD

Background: Medulloblastoma (MB) is the most common pediatric brain malignancy. MB comprises 5 major subgroups known as WNT, SHH p53wt, SHH p53mut, Group 3 and Group 4. Among the four MB subgroups SHH group is the most dominant molecular subgroup in infants and adults. These tumors are proposed to arise from cerebellar granule neuron precursors (CGNPs), whose developmental expansion requires SHH signaling from the neighboring Purkinje neurons. Previous reports suggest that SHH group features a unique tumor microenvironment compared with other MB groups.

Methods: We performed cytokine array analysis of culture media from different SHH group, Patient Tumor cells, spontaneous SHH MB mouse tumor cells and SHH MB cell lines. Further, confirmed these results using ELISA, Western blot, and immunofluorescence from 3 human SHH MB cell lines, Smo/A1 mouse tumor primary cells and PZp53Med cell lines. In continuation to the observation of IGFBP2 expression in various cell types in single cell analysis, we analyzed the presence of IGFBP2 in astrocytes using Smo/A1 mouse tumor Immunohistochemistry.

Results: Our data showed increased levels of IGFBP2 produced by SHH MB cell lines compared to others. We analyzed the role of IGFBP2 in SHH MB tumor growth and metastasis. IGFBP2 knock-down stable cell lines showed phenotypic changes including reduced cell proliferation, cell migration and metastasis. Further western blot analysis of IGFBP2 KD cells showed reduced expression of EMT markers also reduced the activation of STAT3.

Conclusion: Our preliminary in vitro data suggest IGFBP2 exerts it metastasis-promoting role in SHH MB by regulating the expression of EMT marker proteins and matrix remodeling proteins. Further functional studies suggest that in SHH MB, IGFBP2 may regulate a STAT3-mediated EMT program to metastasize. These findings provide a strong rationale for further pursuing how IGFBP2 promotes medulloblastoma tumor cell growth and migration in vivo.
Deep phenotyping of inflammatory proteins after elexacaftor/tezacaftor/ivacaftor therapy in cystic fibrosis

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Presenting Author: Hazel Ozuna, BS, MS, PhD

Cystic Fibrosis (CF) Transmembrane Conductance Regulator (CFTR) modulator therapies have resulted in positive clinical outcomes, yet chronic inflammation and bacterial infections persists. To fill the gap of how elexacaftor/tezacaftor/ivacaftor (ETI) fails to improve bacterial clearance and pro-resolving inflammatory responses we used unbiased proteomics for deep phenotyping of changes in inflammatory proteins before and after ETI. Plasma from 20 people with CF (pwCF) and 20 healthy controls, collected pre- and 3 months post-ETI therapy, was used for targeted protein screening using the Olink® Target 96 Inflammation panel. A total of 92 pre-specified inflammatory proteins were simultaneously measured in plasma samples along with 4 internal controls for quality control. After exclusion of samples that did not pass quality control, 74 out of 92 proteins were detected in >75% of samples. Normalized protein expression units were generated on a log2 scale. Principal component analysis (PCA) was used to identify patterns among CF samples pre-/post ETI and non-CF along with proteomic bioinformatics analyses. Only 30% of recruited pwCF had been on prior CFTR modulators. A year after ETI initiation pulmonary exacerbations were significantly decreased along with significant sustained improvement of lung function (FEV1) and reduced bacterial colonization. No significant changes were observed in nutritional outcomes (BMI z-scores). Normalized values between pre-CF and non-CF were significantly different for all evaluated target proteins. Significantly altered inflammatory proteins were predicted to be involved in cytokine-cytokine receptor interactions, NF-kappa B, chemokine, and IL-17 signaling pathways. PCA and hierarchical clustering showed a shift in ~50% of CF post-ETI samples towards a non-CF profile. Several inflammatory proteins that can serve as CF biomarkers were significantly reduced post-ETI such as IL-17A, IL-6 and MCP-1. In contrast, TRANCE, CASP-8, ADA, IL-4 and NT-3 increased post-ETI. No significant change was observed due to ETI therapy for inflammatory proteins such as IL-8, IL-12B and TWEAK. Overall, ETI therapy demonstrated a modest effect on several inflammatory proteins that could serve as biomarkers of therapeutic response. In contrast, inflammatory proteins unaffected by ETI highlight pathways that could be targeted for development of future CF therapies to combat persisting inflammation.
Pharmacological Modulation of Stemness Pathways to Inhibit the Proliferation of the HIV Reservoir

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Presenting Author: Inna Ruiz-Salinas, PhD, MS

Background: Latently HIV-infected memory CD4+ T cells persist indefinitely through proliferation. We previously showed that inhibition of proliferation and induction of differentiation of central and stem cell memory CD4+ T cells can be achieved in ART-treated SIV-infected rhesus macaques (RMs) through modulation of the Wnt pathway.

Objective: Here, we evaluated a combined approach targeting Wnt and Notch pathways during SIV acute infection of RMs to disrupt viral reservoir establishment.

Design/Methods: The experimental treatment was first evaluated in two uninfected RMs that received 8-week treatment cycles of Wnt inhibitor PRI-724 at 20mg/kg daily and Notch/gamma-secretase inhibitor LY3039478 at 1.5 and 2.5mg/kg three times a week. Five RMs were then infected i.v. with SIVmac239 before receiving PRI-724+LY3039478. ART was initiated 8 weeks post-infection (wpi) and maintained for one year during which PBMC were collected to sort subpopulations of CD4+ T cells by FACS. Cell-associated SIV gag DNA and unintegrated 2-LTR circle levels were measured by multiplex PCR in sorted cells.

Results: The combined treatment PRI-724+LY3039478 demonstrated acceptable pharmacokinetic, pharmacodynamic, and safety profiles in uninfected and SIV-infected RMs. ART successfully suppressed plasma viral load, reaching undetectable levels in 4/5 RMs by 20wpi. During this period, a decrease in circulating CD4+ T cells containing SIV DNA was observed for all memory subsets, but not for the naïve CD4+ T cells. This decay showed a clear biphasic pattern of rapid initial decay followed by a slower decay thereafter in the most differentiated CD4+ T cell subsets. Importantly, the levels of 2-LTR circles that are diluted with proliferation, dropped drastically during the first month of ART specifically in the transitional memory CD4+ T cells.

Conclusion: The Wnt and Notch pathway inhibitors PRI-724 and LY3039478 were safely administered in combination to SIV-infected RMs. The viral reservoir dynamics in these RMs presented distinct characteristics in subsets of peripheral CD4+ T cells. Comparison of SIV DNA levels within CD4+ T cell subsets between this group of RMs treated with PRI-724+LY3039478 and a historical control group treated with ART only will demonstrate whether the combined inhibition of Wnt and Notch pathways alters the viral reservoir distribution.
DFMO inhibits LIN28B and decreases viability of Group 3 Medulloblastoma cells

Authors: Shahab, Shubin; Roggeveen, Christianna; and Kenney, Anna

Presenting Author: Shubin Shahab, MD, PhD

Medulloblastomas, the most common malignant brain tumor in children, are subdivided into 4 major molecular subgroups. Among these, Group 3 medulloblastomas have one of the worst prognoses. We have recently identified the LIN28B pathway as being critically important in Group 3 medulloblastoma growth in vitro and in vivo. There are few targeted inhibitors of the LIN28B pathway and none are currently in clinical practice. Recently DFMO has been identified as an indirect inhibitor of the LIN28B-let-7 axis through its inhibition of polyamine biosynthesis. Another recent study identified ornithine, a precursor to polyamines, as being upregulated in MYC amplified Group 3 medulloblastoma. Importantly, DFMO has been demonstrated to achieve high concentrations in brain tumors in a rat glioma model with minimal systemic toxicity. Based on this we hypothesize that DFMO would be an attractive agent for Group 3 medulloblastoma treatment as it would inhibit both polyamine synthesis and LIN28B and potentially achieve high tumor concentrations. Here we demonstrate that treatment with increasing concentration of DFMO leads to significant reduction in Group 3 medulloblastoma viability, increased apoptosis and reduced levels of LIN28B and its downstream targets. We are currently investigating whether this drug can effectively inhibit in vivo tumor growth in orthotopic xenograft models of Group 3 medulloblastoma.
Repolarizing the Tumor Microenvironment in Osteosarcoma with Interferon-gamma Secreting Mesenchymal Stromal Cells to Promote Immunotherapy

Authors: Smart, Sherri; Foppiani, Elisabetta; and Horwitz, Edwin

Presenting Author: Sherri Smart, MD, PhD

Background: Osteosarcoma is the most common bone tumor in children and adolescents, and outcomes remain poor in patients with metastatic, relapsed, or refractory disease. Macrophages located in the tumor microenvironment, or tumor associated macrophages (TAMs), polarized towards an M1 phenotype are pro-inflammatory / anti-tumor, while those polarized towards an M2 phenotype are considered anti-inflammatory / pro-tumor. Interferon-gamma (IFN-γ) is a signal secreted by T cells and other immune cells which polarizes macrophages to an M1 phenotype. Previously we have shown that genetically modified mesenchymal stromal cells (MSCs) secreting IFN-γ promote M1 polarization and decrease tumor volume in vivo in neuroblastoma mouse models. We hypothesize that treatment with IFN-γ secreting MSCs in osteosarcoma will induce M1 macrophage polarization and a pro-inflammatory tumor microenvironment.

Objective: To evaluate interferon-gamma secreting mesenchymal stromal cells as a novel therapy in osteosarcoma

Design/Methods: MSCs were genetically modified through transduction of lentiviral particles containing IFN-γ or GFP control. Studies are ongoing in immune competent, cell-lined derived mouse models of osteosarcoma to determine the effect on tumor burden upon treatment with IFN-γ secreting MSCs compared to control MSCs and PBS. Alterations in cytokines and chemokines related to pro-inflammatory / anti-tumor (M1) and anti-inflammatory / pro-tumor (M2) phenotypes in the tumor microenvironment are being characterized using magnetic Luminex assays.

Results: Our laboratory has shown that treatment with mIFNγ secreting MSCs polarizes primary bone marrow derived macrophages to an anti-tumor M1 phenotype, with increased expression of M1 markers IL-6, CD38, and NOS2 and decreased expression of M2 markers IL-10 and Mrc-1. Studies are ongoing determining the antitumor effects in vivo of IFN-γ secreting MSCs in mouse models of osteosarcoma.

Conclusions: We are utilizing IFN-γ secreting MSCs to polarize TAMs within the osteosarcoma tumor microenvironment to the pro-inflammatory / anti-tumor phenotype. If IFN-γ secreting MSCs do not decrease tumor growth in vivo, therapeutic effects of MSCs secreting other immune modulators (eg IL-12, IL-2) will be examined. These preclinical experiments provide the first step in translating this therapy into patients, with the goal of ultimately improving outcomes and quality of life for patients with osteosarcoma.
Disruption of the serine-producing pathways slows progression of the Sonic Hedgehog-driven medulloblastoma

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Presenting Author: Andrey Tikunov, PhD

We have found that medulloblastoma metabolism is specialized to promote growth in the CNS and can be targeted for anti-tumor therapy. Prior studies show that serine is scarce in the CNS and that metastatic tumors require serine-producing enzymes to grow in the brain. We analyzed whether medulloblastoma, a primary brain tumor, showed similar serine dependency. We found that Sonic Hedgehog signaling, which induces proliferation in cerebellar granule neuron progenitors in normal development and medulloblastoma formation when hyperactivated, also induces PHGDH and SHMT1, which each catalyze key steps in different serine-production pathways. To define the functional role of PHGDH and SHMT1 in medulloblastomas, we bred mice genetically engineered to develop SHH medulloblastomas with mice carrying deletions of each of these genes. We found that mice with Phgdh-deleted medulloblastomas showed slower progression and longer survival times, compared to mice with Phgdh-intact medulloblastomas. Medulloblastomas with combined deletion of Phgdh and Shmt1 show even slower tumor progression. Stable-isotope flux analysis suggests that different serine-producing pathways compensate for the loss of individual serine-producing enzymes, providing a rationale for the greater anti-tumor effect of disrupting multiple serine-producing enzymes simultaneously. Our data show that serine-producing enzymes can be targeted as a novel approach to medulloblastoma therapy, and underscore the importance of targeting multiple serine-production pathways in complex metabolic interventions.
Inducing Stenotic Hemodynamics in an In Vitro Bioprinted Pulmonary Vein Mimic Induces Rapid Endothelial to Mesenchymal Transition (EndMT) in Endothelial Cells.

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Presenting Author: Martin Tomov, PhD

Background: Pulmonary Vein Stenosis (PVS) is an acute cardiovascular condition characterized by progressive lumen size reduction due to overgrowth of connective and fibrotic tissue in one or more of the pulmonary veins. While current clinical interventions (stenting and angioplasties) have shown promising results in treating PVS, they require multiple re-interventions that can lead to re-stenosis and diminished long-term efficacy. There is an unmet need to develop functional in vitro models of PVS that can serve as a platform to study clinical interventions, specifically addressing endothelial to mesenchymal transition (EndMT) and flow shear stress changes, which are major triggers for stenosis and restenosis. We thus hypothesize that patient-inspired 3D bioprinted tissue models can provide a unique approach to recapitulate changing shear stress and EndMT transitions and allow for analysis of the complex tissue microenvironment impacted during PVS.

Methods: We developed perfusable in vitro models of healthy and stenotic pulmonary vein by 3D reconstruction and bioprinting of patient-inspired computer tomography and X-ray angiography data. Flow hemodynamics through bioprinted vein models were predicted via computational fluid dynamics modeling and measured experimentally using 3D ultrasound particle imaging velocimetry (PIV). These PVS models were then seeded with human endothelial cells and assayed for cell survival, growth, and cell states within the printed channels while under homeostatic flow in either healthy or stenotic bioprinted geometries.

Results: Our work here successfully demonstrated a how to generate perfusable and cellularized biomimetic constructs that can be used to model complex biological processes. The constructs further allowed us to incorporate and quantify the effects of tissue-like geometrical, chemical, metabolic and biomechanical ques that could offer substantial insights for prevention and treatment of PVS, as well as other cardiovascular diseases.

Conclusions: Our work demonstrates the feasibility of bioprinting perfusable, patient-inspired, cardiovascular constructs that can recapitulate complex in vivo structures, survive long-term under homeostatic flow rates, and recapitulate functional aspects of the stenosis triggers, namely EndMT cascades.
Elucidating the effects of combination therapy with Venetoclax and IAP inhibitor AZD5582 in SIV-infected, ART-suppressed macaques

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Presenting Author: Benedict Ukhueduan, BS, BAS, PhD

Background: Combining the IAP inhibitor AZD5582 with the Bcl-2 inhibitor Venetoclax (ABT199) to reverse latency and enhance clearance of infected cells via apoptosis is a novel approach to cure HIV. In this pilot study, we evaluated the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of ABT199 with AZD5582 in a nonhuman primate model.

Methods: Two juvenile rhesus macaques (RMs) were orally infected with SIVmac251 and ART was initiated 4 wpi. At 112 wpi, RMs received escalating single doses of ABT199 at 2.5 mg/kg, 10 mg/kg and 20 mg/kg intramuscularly (i.m.). Next, AZD5582 was administered once intravenously at 0.1 mg/kg with 4 daily doses of ABT199 (at 15 mg/kg i.m.). PK parameters in plasma was evaluated and changes in absolute T- and B-cell counts were assessed by flow cytometry.

Results: Venetoclax was well tolerated, with only mild adverse events (AEs) at 10 and 20 mg/kg single doses and no AEs with repeated doses given in combination with AZD5582. AEs included short term injection site pain and swelling, with nausea and vomiting in 1 RM (20 mg/kg dose). Steady state Cmax after 15 mg/kg i.m. ABT199 dose 4 (2.26 μg/ml) approximates the Cmax in humans after 400 mg oral dosing (2.1+/-1.1 μg/ml), with similar AUC0-24 but shorter T1/2 observed. Absolute CD20+ B-cell counts were used as a PD marker, with reduction of 62-91% found at 24h post ABT199 dose 1 (15 mg/kg) + AZD5582 and full recovery to baseline levels by 2 weeks post ABT199 dose 4. Absolute CD4+ and CD8+ T-cell counts also declined at 24h post ABT199 dose 1 + AZD5582, including both naïve and memory subsets, without complete recovery by 2 weeks post ABT199 dose 4.

Conclusions: Venetoclax can be safely administered to RMs alone and in combination with the AZD5582. An immunologic response was achieved, including depletion of memory and naïve CD4+ T-cells, supporting the use of Venetoclax as a strategy to clear viral reservoirs. An efficacy study to evaluate latency reversal and apoptosis of infected CD4+ T-cells using combination therapy with AZD5582 and Venetoclax in ART-suppressed RMs is underway.
Are children with sickle cell disease at particular risk from the harmful effects of air pollution? Evidence from a large, urban/peri-urban cohort.

Authors: George, Paul; Maillis, Alexander; Zhu, Yijing; Liu, Yang; Lane, Peter; Lam, Wilbur; Lipscomb, Joseph; and Ebelt, Stefanie

Presenting Author: Paul George, MD

Background: Key pathophysiologic pathways of both sickle cell disease (SCD) and air pollution exposure include increased inflammation, oxidative stress, and endothelial damage. It is therefore plausible that children with SCD are especially prone to harms from air pollution. We aimed to determine if increases in citywide, daily ambient (outdoor) pollutant levels were associated with more emergency department (ED) visits among a large cohort of children with SCD and a comparison group of children without SCD.

Methods: Patient data were retrospectively collected from a single pediatric hospital system in Atlanta, GA, forming an urban/peri-urban cohort of children with confirmed SCD and a comparison group of children without SCD, ages 1-17. Daily ambient concentrations of fine particulate matter (PM2.5) were collected via NASA satellite-derived remote-sensing products, and carbon monoxide (CO), nitrogen dioxide (NO2), and ozone were collected from local monitoring stations. We used multivariable negative binomial regression to quantify associations of pollutant levels (main exposure variable) with daily counts of ED visits (main outcome variable), accounting for weather and time trends.

Results: From 2010-2018, there were 17,731 ED visits by 1740 children with SCD (64.8% HbSS/HbSβ0). Vaso-occlusive events (57.8%), respiratory illness (17.1%), and fever (16.1%) were most common cause of visits. Three-day (lags 0-2) rolling mean PM2.5 and CO levels were associated with daily ED visits among those with SCD (PM2.5 incident rate ratio (IRR) 1.051 (95% CI 1.010-1.094) per 9.4 µg/m3 increase; CO 1.088 (1.045-1.132) per 0.5 ppm). NO2 showed positive associations in secondary analyses; ozone levels were not associated with ED visits. The comparison (non-SCD) group demonstrated lower IRR for all four pollutants. Our models were robust to various distributional assumptions, different time and time-trend lags, and 1- and 2- day lead analyses.

Conclusions: Our results suggest that increases in short-term ambient air pollution levels may be potential triggers for SCD events and that children with SCD may be more vulnerable to harms from air pollution than those without SCD. Targeted pollution-avoidance strategies could have significant clinical benefits in this population.
Low arginine bioavailability and clinical outcomes in children with sickle cell disease (SCD) hospitalized with vaso-occlusive pain episodes (VOE)

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Presenting Author: Dunia Hatabah, MD

Introduction: Global arginine bioavailability ratio (GABR) (Arginine/[Citrulline+Ornithine]) is associated with clinical outcomes including pain, pulmonary hypertension risk and mortality in SCD. Arginine replacement therapy demonstrates opioid-sparing effects, improved blood pressure and cardiovascular function, and shorten length of hospital stay. We have previously shown that patients with SCD-VOE have an altered arginine metabolome associated with pain severity. Arginine is a conditionally essential amino acid synthesized from citrulline in the kidneys. During VOE, hemolysis leads to the release of erythrocyte-arginase (arginine-metabolizing enzyme) that metabolizes arginine into ornithine. Arginine-to-ornithine ratio (arg/orn) is a biomarker of arginase activity, while GABR incorporates the impact of kidney function on global arginine bioavailability. The association between arginine bioavailability and clinical outcomes has not been sufficiently explored in children with SCD-VOE.

Objective: To evaluate associations between arginine bioavailability and clinical outcomes in children with SCD-VOE receiving intravenous (IV) arginine replacement therapy (ART).

Methods: Prospective single-center double-blind randomized controlled trial (RCT) of IV ART (TID, up to 7 days) in children with SCD age 3-21 years hospitalized for VOE requiring IV opioids. Patients with significant liver/renal dysfunction or those previously enrolled were excluded. Subjects were randomized into 1 of 3 arms: 1) 100 mg/kg/dose Arg, standard dose (SD), 2) loading dose: 200 mg/kg followed by SD or 3) placebo. Demographics, total parenteral opioid (TPO) use (morphine equivalents, mg/kg) time to crisis resolution (time of study drug delivery to last IV opioid in hours), pain scores and targeted amino acids were obtained before treatment & at discharge. A biorepository for future mechanistic studies was also created. Mean±SD, paired t-tests and Pearson correlation analyses between groups were performed where appropriate.

Results: Safety results of this RCT have been previously reported (Reyes et al, Am J Hematol 2022). Plasma arginine levels were low at VOE presentation (mean 50±28 μM), with low arginine levels (12, subjects >12 years old showed a strong negative correlation between plasma arginine levels and time-to-crisis resolution (r=-0.5, p=0.02) in the placebo group only. Pain scores at presentation positively correlate with TPO (r=0.33, p=0.04) in the placebo arm only, which was lost after arginine therapy (r=0.03, p=0.76).

Conclusion: In the absence of ART, low arginine bioavailability predicts worse clinical outcomes in patients with SCD-VOE, including a longer time-to-crisis resolution and a higher use of IV opioids. Arginine bioavailability may represent a novel biomarker of SCD-pain severity. This data further confirms arg/orn as a surrogate for arginase activity reflective of hemolytic rate as previously described. ART ameliorates the influence of arginine deficiency on clinical outcomes related to pain in SCD. An NHLBI/PECARN supported Phase-3 multi-center trial of ART enrolling 360 children with SCD (SCD Treatment with Arg Therapy – STArT) is ongoing.
Bedside Cardiac Ultrasound in the Pediatric CICU: The Modern Physical Exam

**Authors:** Mills, Marcos; and Maher, Kevin

**Presenting Author:** Marcos Mills, MD

Background: Despite a multitude of invasive and non-invasive methods of monitoring patient hemodynamics in the pediatric cardiac intensive care unit (CICU), patients remain at risk for clinical decompensation. It is unknown whether the use of portable focused cardiac ultrasound (FCU) as part of the routine physical exam would result in the discovery of new information in a timely manner that meaningfully adds to the clinical evaluation.

Methods: In this prospective descriptive study at Children's Healthcare of Atlanta's pediatric cardiac intensive care unit, cardiac intensivists with board certification in pediatric cardiology performed FCUs on both post-operative and non-surgical critically ill patients. Successful FCU completion consisted of the acquisition of 4 imaging windows: parasternal long, parasternal short, apical, and subcostal. Pre and post-FCU questionnaires were filled out comparing echocardiographic estimates (pre) to echocardiographic findings (post), along with physician experience and impact on clinical management.

Results: Six cardiac intensivists performed 34 FCUs. 62% of the encounters occurred with patients who had previously undergone cardiac surgery during their hospitalization, which was less than 48 hours prior to FCU in 25 (74%) of these encounters. The majority of FCUs (74%) were completed in less than 3 minutes and with the successful acquisition of all imaging windows in 88% of encounters. The median time since their most recent formal echocardiogram was 4 days (IQR 2.25-7). New findings occurred in 59% of FCUs and resulted in changes in clinical management 18% of the time. Physician confidence in their clinical assessments increased in 50% of encounters.

Conclusions: In this novel use of portable ultrasound technology, pediatric cardiac intensivists frequently discovered new information that would not otherwise have been known at that moment and often resulted in changes to clinical management. The technology was easily incorporated and frequently increased overall physician confidence in their clinical assessments. Further investigation regarding the use of portable focused cardiac ultrasound’s potential impact on clinically relevant outcomes is warranted.
Derivation and Validation of a Novel Risk Assessment Tool to Identify Young Children at Risk for Post-Discharge Mortality in Dar es Salaam, Tanzania and Monrovia, Liberia

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Presenting Author: Chris Rees, MD, MPH

Background: Rates of post-discharge mortality among children in sub-Saharan Africa are as high as 3-18% within months of hospital discharge versus <1% among children in the US. Accurately identifying infants and young children at risk for post-discharge mortality is a crucial step towards reducing mortality in this period of vulnerability following a hospitalization. Our objective was to derive and internally validate a risk assessment tool to identify infants and children at risk for all-cause mortality within 60 days of discharge from the pediatric wards of two referral hospitals in Dar es Salaam, Tanzania and Monrovia, Liberia.

Methods: We performed a prospective observational cohort study of infants and children aged 1-59 months who were discharged from pediatric wards at Muhimbili National Hospital in Dar es Salaam, Tanzania and John F. Kennedy Medical Center in Monrovia, Liberia from October 2019 to January 2022. Caregivers of enrolled participants received phone calls at 7, 14, 30, 45, and 60 days after discharge to ascertain participants’ vital status. We collected socioeconomic, demographic, clinical, and anthropometric data for each enrolled participant during their hospital admission. We included candidate variables with a P value <0.20 in bivariate analyses into a multivariable logistic regression model with best subset selection to identify risk factors for all-cause, 60-day post-discharge mortality. We selected the model with the lowest Akaike Information Criterion and Bayesian Information Criterion as the final model. We calculated adjusted log coefficients for each candidate variable and assigned weighted points to derive our risk assessment tool. We used bootstrap validation with 500 repetitions to internally validate our tool.

Results: There were 2,047 infants and children discharged. 1,933 (94.4%) enrolled and 1,895 (98.0%) had 60-day outcomes available. The median (interquartile range) age at discharge was 11 (4, 23) months and 1,135 (59.8%) were male. In total, 67 (3.5%) died within 60 days of discharge. While cancer, pedal edema at discharge, leaving against medical advice, and cyanosis were most predictive of post-discharge mortality, 10 variables contributed to our tool for a total possible score of 82. A score of ≥11 was the optimal cut point to identify infants and children at risk for post-discharge mortality. Our risk assessment tool demonstrated good discriminatory value (optimism corrected area under the receiver operating characteristic curve 0.77) and excellent calibration.

Conclusions: Our risk assessment tool to predict all-cause, 60-day post-discharge mortality among infants and children in Tanzania and Liberia had good discriminatory value. Risk assessment tools, such as this, must be externally validated before implementation. After validation, this tool may be used in sub-Saharan Africa to identify young children at risk for post-discharge mortality and direct focused resources and follow up for high-risk children.
Distinguishing MIS-C Features from Febrile Patients Evaluated for Kawasaki Disease Pre-Pandemic

**Authors:** Bass, Marissa; Fan, Lucie; Jaggi, Preeti; and Sherry, Whitney

**Presenting Author:** Marissa Bass, MD

**Background:** The distinction between Kawasaki Disease (KD), Multisystem Inflammatory Syndrome in Children (MIS-C) and alternate diagnoses has become complex given the overlap of clinical manifestations of KD in the definition of MIS-C compounded by increasing COVID-19 antibody positivity. We sought to determine the clinical characteristics and final diagnoses of febrile patients who were evaluated for Kawasaki disease (EVAL KD), but not diagnosed with or treated for KD, from prior to the COVID-19 pandemic in 2018. We also sought to identify clinical features of non-severe MIS-C that distinguish this novel diagnosis from other potentially confounding diagnoses.

**Methods:** This descriptive study compared demographic, clinical, and laboratory features of two cohorts. We included children hospitalized for possible KD with ≥2 KD criteria, or who had an echocardiogram for concern for KD in the EVAL KD group (1/2018-12/2028). We excluded children without fever or who received intravenous immunoglobulin. We defined the non-severe MIS-C cohort (3/2020-2/2021) using the 2023 CDC case definition and excluded children requiring intensive care treatment.

**Results:** The MIS-C cohort (n=68) was older (3 vs 8-years-old, p<0.001), more likely to have conjunctival injection (42% vs 74%, p<0.001), multi-organ system involvement (p<0.001), lymphopenia (p<0.001) and thrombocytopenia (p<0.001); there was no significant difference in presence of anemia (p=0.253). Cardiac involvement was significantly higher in the MIS-C cohort. None of the EVAL KD group (n=81) had depressed cardiac function vs. 51.5% of the MIS-C cohort had either depressed function, coronary involvement, or an elevated troponin (p<0.001). The EVAL KD group was more likely to have a viral infection (p<0.001); adenovirus was the most common virus (11%). Twenty-one percent of the EVAL KD cohort did not have a conclusive final diagnosis.

**Conclusions:** Viral infection, mostly adenoviral, remains a common diagnosis prompting evaluation for KD. Age, organ system involvement, and CBC can be useful in identifying children more likely to have MIS-C when admitted for fevers without a source and mucocutaneous findings.
Evaluating the Association Between Structural Racism and Acuity of Illness at Initial Presentation in Pediatric Patients with Solid Tumors

Authors: Cathcart, Alexandra; Castellino, Sharon; Sohn, Heeju; DeGroote, Nicholas; Mertens, Ann; and Ji, Xu

Presenting Author: Alexandra Cathcart, MD

Background: There is growing recognition regarding racial disparities in pediatric cancer outcomes, despite overall improvements in disease-free survival. Pediatric patients depend on families’ ability to bring them to medical attention and to manage their cancer; this is largely determined by their communities’ social, economic, and environmental factors. Patients’ acuity of illness at initial presentation therefore may reflect structural factors in communities and can ultimately be linked to morbidity and mortality. Furthermore, initial acuity of illness may be linked to structural racism in patients’ residential communities. Understanding and quantifying structural racism – racial/ethnic disparities embedded in disadvantaged neighborhoods – is a critical step toward delineating the modifiable factors that underlie disparities in pediatric cancer outcomes.

Methods: This is a retrospective, single institutional-based study of N=1149 pediatric patients treated at Children’s Healthcare of Atlanta from 2010 to 2018 who were diagnosed with a solid malignancy. The cohort is being linked to a county-level structural racism index (SRI), which innovatively integrated neighborhood deprivation and racial residential segregation using patient addresses at diagnosis. Acuity of illness based on the need for ICU-level resources was identified through medical record abstraction. Differences in sociodemographic characteristics will be analyzed by the SRI levels using chi-squared, Student t-tests, and ANOVA as appropriate. Logistic regression models will be used to assess the association of the SRI with acuity of illness.

Results: To date, 353 records have been abstracted. We anticipate that relevant sociodemographic characteristics will differ by SRI levels once data are completely abstracted. Furthermore, we anticipate that patients living in counties with the highest SRI quartile (the greatest level of structural racism) are more likely to have a high acuity of illness at initial presentation, compared to those living in counties with the lowest SRI quartile.

Conclusions: By applying a novel index quantifying structural racism to a cohort of pediatric patients with solid tumors, findings of this study will provide new information about the structural barriers patients face at the time of diagnosis. This work will allow us to identify areas for future interventions in community care, resources during treatment, and health care policy in this vulnerable patient population.
Mortality and outcomes of children with tracheostomy before and after implementing high-fidelity simulation-based tracheostomy education

Authors: Fain, Mary Ellen; Prickett, Kara; Jergel, Andrew; Gillespie, Scott; and Kasi, Ajay

Presenting Author: Mary Ellen Fain, MD

Background: Children with tracheostomies (CwT) experience elevated mortality rates. Tracheostomy-related accidents (TRA – mucus plugging, accidental decannulation, and hemorrhage) contribute significantly to overall outpatient mortality and are preventable. High-fidelity simulation-based tracheostomy education (SBT) using a programmable mannequin to simulate tracheostomy-related emergencies was implemented in 2017 to educate family caregivers on the outpatient management of urgent situations and TRAs. Outcomes for CwT before and after implementation of SBT are unknown. Our study aimed to assess mortality and frequency of emergency room (ER) visits and hospitalizations in CwT before and after implementing SBT.

Methods: A retrospective cohort study of children aged <18 years who underwent tracheostomy at our institution in 2016 (pre-SBT) and 2018 (post-SBT) was conducted. The medical records were reviewed for one year after hospital discharge following tracheostomy placement. The pre-SBT and post-SBT cohorts were compared to assess mortality and frequency of ER visits and hospitalizations. Analyses included descriptive statistics, Fisher’s exact test, and Wilcoxon rank-sum test.

Results: Among 43 CwT during the study period, the caregivers of 18 CwT completed SBT. There was no difference in the median (IQR) age at tracheostomy between the pre-SBT [0.84 (0.34-8.11) months] and post-SBT groups [0.46 (0.32-1.3) months] (p=0.14). Although not statistically significant, the one-year mortality was higher in the pre-SBT group (20%) compared to the post-SBT group (0%) (p=0.06). There was 1 death due to TRA in the pre-SBT group and none in the post-SBT group (p=1.0). There was insufficient evidence to conclude that the two groups differed based on the total number of ER visits (p=0.91) and ER visits for tracheostomy-related problems (p=0.24) in one year. Similarly, there was insufficient evidence to conclude that the two groups differed based on the total number of hospitalizations (p=0.37) and hospitalizations for tracheostomy-related problems (p=1.0) in one year.

Conclusions: Our study showed that SBT for caregivers of CwT may reduce overall mortality as well as mortality due to TRA. Although CwT required several ER visits and hospitalizations, there was no difference in the frequency of ER visits and hospitalizations between those who did and did not complete SBT.
The Utilization of Vasopressor Infusions during Pediatric Liver Transplantation and Association with Postoperative Outcomes

Authors: Bartels, A; Karlik, J; Liu, K; and Gilbertson, L

Presenting Author: Laura Gilbertson, MD

Background: Pediatric patients undergoing liver transplantation frequently require vasopressor infusions. While vasopressors may have a detrimental effect on end-organ or graft perfusion, there is limited data on the effect of intraoperative vasopressor use on postoperative outcomes in pediatric liver transplant patients.

In this retrospective chart review, we aim to determine the post-operative effects of perioperative vasopressors during pediatric liver transplantation. We hypothesized that perioperative vasopressors result in untoward outcomes such as increased length of stay, infection, and graft dysfunction.

Methods: Following IRB approval, we evaluated the records of all patients who underwent a liver transplant at our campus from January 1, 2014 to June 1, 2021. The vasopressor group was characterized as any patient who had a vasopressor infusion for a minimum of 15 consecutive minutes intraoperatively. End points included re-exploration, re-transplantation, post-op infection, length of stay, and mortality.

Results: 173 patients were analyzed, with 134 (77.5%) meeting criteria for the vasopressor infusion group. Baseline characteristics were similar between the two groups with the exception of patients presenting from home were less likely to require a vasopressor infusion. The no vasopressor infusion group had significantly more patients who were able to be extubated post-op. The other significant difference was in LOS, which was shorter in the no vasopressor infusion group. When broken down by type of infusion, the use of a norepinephrine resulted in significantly longer LOS than any other type of vasopressor (5.58 days). Also, the total number of infusions used had no effect on outcomes.

Conclusion: The intraoperative management of pediatric liver transplantation frequently requires vasopressor use. Often, the utilization of these infusions is limited to the intraoperative course, but prior to this study there was limited data on the postoperative effects. Our results show that compared to no vasopressor use, the use of any number of vasopressors confers an increased risk of length of stay, particularly when using norepinephrine. However, the use of any number of vasopressors has no effect on postoperative outcomes such as infection and graft dysfunction. Further evaluation looking at the length of intraoperative vasopressor infusion and its effect on outcomes would be beneficial.
Longitudinal Surveillance of SARS-CoV-2 (SARS) IgG Antibodies in Pediatric Healthcare Workers (HCW)

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Presenting Author: Dunia Hatabah, MD

Background: Vaccines against SARS target the spike protein. There is minimal information on longitudinal immune profiling of different subgroups including SARS recovered vs. naïve (never infected with SARS) individuals and vaccinated vs. non-vaccinated in HCW.

Objective: Explore the impact of SARS vaccination on IgG antibody titers over time and cross-reactivity with other corona viruses in a longitudinal cohort of HCW.

Methods: HCW were enrolled in this prospective longitudinal cohort to determine Covid-19 vaccine triggered IgG titers over time post vaccination series and post booster.

Results: 642 HCW were enrolled with a 4% prevalence of SARS-IgG & 8% incidence of new infection from Sept-Dec 2020. 354 participants had repeat IgG titers measured at different time intervals for post Covid-19 vaccine titers. A robust antibody response occurred against RBD (receptor-binding domain), spike and NTD (N-terminal domain) in all vaccinated individuals vs. non-vaccinated (p<0.0001). COVID-19 recovered-vaccinated participants higher titers of spike and RBD than naïve individuals after vaccination (p<0.0001). Recovered participants showed higher IgG titers for other beta-corona variants irrespective of vaccination status. Single dose of vaccine was sufficient to attain maximum titer in Covid-19 recovered participants compared to naïve that required both doses of vaccine. RBD and Spike antibody titers were higher and more durable after booster as compared to primary series of vaccination.

Conclusion: All vaccinated HCW developed SARS-IgG to spike. Both SARS infection and vaccination yield antibodies that cross react to other beta-corona viruses, likely imparting additional immunity against different strains. RBD and Spike antibody titers were higher and more durable after booster as compared to primary series of vaccination. Longitudinal profiling of the immune response to vaccination may be useful for counseling future vaccination booster requirements.
Association of Age with Acuity and Severity of Illness at Initial Presentation in Children, Adolescents, and Young Adults with Leukemia

Authors: Jain, Tarun; Ji, Xu; Himes, Alexandra; DeGroote, Nicholas; Coxhead, Cortland; Mertens, Ann; and Castellino, Sharon M.

Presenting Author: Tarun Jain, MD

Background: Adolescent and Young Adult (AYA) patients diagnosed with cancer at ages 15-39 years often have increased morbidity and mortality compared with younger patients, partly due to differences in cancer biology and access to healthcare resources. Acuity and severity of illness at presentation and its role in morbidity and mortality has been understudied in this vulnerable population.

Objective: To characterize the acuity and severity of illness at initial presentation of leukemia by patient age.

Design/Methods: We performed a retrospective analysis of a cohort of patients diagnosed with leukemia in 2010-2018 at Children's Healthcare of Atlanta. We abstracted data on intensive care unit (ICU) resource use in the first 72 hours of care, white blood cell (WBC) count, and central nervous system disease from medical records. Based on treatment classification of “high-risk” leukemia, age was categorized into two groups: 1-9 years; 10-21 years. Bivariate comparisons were performed on the data abstracted to date.

Results: Patients aged 10-21 had a higher odds of cardiovascular resource use (OR 7.59, 95% CI 1.58-36.38) and WBC count equal to or greater than 50,000 cells/microliter (OR 1.85, 95% CI 1.09-3.14) than patients aged 1-9. The odds of ICU admission, respiratory, hematologic, renal, and neurologic resource use were not statistically different across age groups.

Discussion/Conclusions: These preliminary results set the stage for continued data abstraction for the entire cohort, as well as for multivariable analysis that will test the association of acuity and severity of illness with age, adjusting for health insurance status, race, ethnicity, and local-area social vulnerability. Our findings will help inform strategies toward narrowing age disparities in outcomes of AYA hematologic cancers.
Incidence, Risk Factors, and Outcomes of Postreperfusion Syndrome in Pediatric Liver Transplantation

Authors: Joseph, Ashley; Cucino, Matthew; Ji, Dabin; Gilbertson, Laura; Tolly, Renee’; and Fiedorek, Michael

Presenting Author: Ashley Joseph, MD

Introduction: Postreperfusion syndrome (PRS), defined by acute blood pressure, heart rate, and vasoplegic changes, is a potentially lethal intraoperative complication during pediatric liver transplantation (PLT) with a variable reported incidence. Our purpose was to determine the incidence, clinical characteristics, risk factors, and associated outcomes of PRS in our PLT population.

Methods: This single-center retrospective study evaluated PLT patients from 4/30/14 - 10/8/21. Wilcoxon rank sum tests were used for nonparametric continuous variables. Categorical variables were compared using chi-squared and Fisher’s exact tests. Logistic regression models were created from the univariate analysis. Results were analyzed with R software (version 4.2.0). PRS occurred in 13% of patients within 5 minutes of reperfusion. Vasopressor infusion(s) was initiated for sustained postreperfusion hypotension in 61 (45%) patients. The variables found to be independent predictors of PRS are noted in Table 2. PRS was not found to be a risk factor for hepatic artery or portal venous thrombosis, re-exploration, infection, PICU or hospital LOS, re-transplant, or death.

Discussion: During reperfusion of the donor liver graft, pediatric patients often experience hemodynamic alterations known as PRS. We identified many clinically significant independent risk factors for PRS, including the severity of cooling after reperfusion, hemodynamic instability before reperfusion, pRBC transfusion, and higher serum potassium. We also found that both female sex and higher hematocrits were independently protective, the latter of which concurs with the Zhang study. The occurrence of PRS was not associated with post-operative morbidity or mortality.

Conclusions: Our study identified the incidence of and risk factors for PRS in the PLT population. There was no association between PRS and postoperative morbidity or mortality.
Steroid-Associated Adverse Events are Dose-Dependent Following Hematopoietic Cell Transplantation for Pediatric Hematologic Malignancies

Authors: Lisac, Robert; Raghunandan, Sharmila; Gillespie, Scott; Liu, Katie; Williams, Kirsten; Watkins, Benjamin; and Qayed, Muna

Presenting Author: Robert Lisac, MD

Background: Hematopoietic cell transplantation (HCT) is a successful treatment of many hematologic malignancies, but can be associated with high systemic steroid exposure (SSE) due to treatment of graft-versus-host disease (GVHD). Steroids are associated with many late complications including poor bone health and cataracts. The objective of this study was to enumerate avascular necrosis (AVN), fractures, and cataracts following pediatric HCT for hematologic malignancies and examine the association of post-HCT SSE with the incidence of these toxicities.

Methods: In this retrospective study, pediatric recipients of first HCT for hematologic malignancy at a single institution between 2011 to 2019 and survived at least 100 days post-HCT were captured. Exclusions from subset analysis included: pre-existing AVN, fractures, and cataracts. Patients were censored at graft failure, relapse, and death.

Results: One hundred thirty-three patients were identified with a median age of 11 years old (range 0.5 - 21) at HCT. Ninety-three (70%) patients had post-HCT SSE, 89 (67%) had acute and 55 (41%) had chronic GVHD. Median SSE was 53 mg/kg prednisone equivalents (PE) (IQR 0 - 186). Twenty-two (19%) patients experienced bone events, including AVN (n=9) and fractures (n=13). Median time to bone event was 1.6 years (range 0.3 - 5.3) post-HCT. The 7-year cumulative incidence rate of bone events was 51% (95% CI 32% - 69%) if exposed to >115 mg/kg PE versus 11% (95% CI 5% - 22%) if ≤115 mg/kg PE (p=0.015). On multivariate analysis, cumulative SSE remained significantly associated with bone events and cataracts.

Conclusion: Our results suggest that adverse bone events and cataracts are common in pediatric patients with hematologic malignancies following HCT and are increased in patients who received higher SSE. These results reinforce that patients should be closely followed for late complications of SSE and highlights the need for steroid-sparing approaches to GVHD treatment.
An Educational Intervention for Caregivers of Children with Motor Delay on a Social Media Platform: Parent Recruitment and Engagement

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Presenting Author: Larken Marra, PhD

Background: Children born with motor delays often miss critical opportunities to improve their motor trajectory due to delays in obtaining specialized care. It may be possible to improve parent self-capacity and motor outcomes for children by empowering parents through knowledge of motor development, principles of infant motor learning, parenting and parental self-care. To address these goals, we designed a program based on caregiver-skills models and delivered on a social media platform over 9-weeks. Here we report on caregiver characteristics and perception of the program.

Methods/Study Design: Randomized trial with waitlist control. Included: parents of children with motor delay < 3 years of age. Exclusion: Non-English speaking or non-U.S. resident. Parents completed mother-infant bonding (MIB), maternal self-efficacy (MES), and caregiver knowledge (CK) of motor skill development questionnaires. Motor delay of the child was quantified using DAYC-2 motor scales. For those who completed the study to date, subjective impression of the intervention was obtained on Likert scales and open-ended feedback.

Results: Of 59 consented and 48 completed initial assessments. Most participants were mothers (91.7%) and Caucasian (79%), with 79% having a 4-year college degree or higher. Their children at study start had a mean of 15 months (SD 9); 44% born preterm with mean DAYC scaled scores of 84.5 (SD 13.4) and 69.1 (SD 15.9) in fine and gross motor domains respectively. Mean MIB and MSE scores were 2.69 (SD 3) and 72.7 (SD 6.5), indicating well-bonded parents who had moderate self-efficacy. Mean CK scores, 20.9 (SD 1.8), revealed a general understanding of motor skill development, with gaps in how infants learn, positive parenting strategies, and parenting self-care. Analysis of the program feedback revealed three themes that parents saw of value in the intervention: more effective parental support for children, tailored parental support by study team, and sense of belonging to a community.

Conclusion: Parents of children with motor delays who chose a social media support program may be well-bonded with their children but had opportunities to increase their self-efficacy and knowledge of infant learning. Opportunities for future improvement of the program include limiting repetitive content, enhancing the quality of embedded videos, and increasing participant interaction on the social media platform.
Positive Bacterial Blood Cultures and Time to Positivity in Children: Should Empiric Antibiotics be Reconsidered Sooner?

Authors: Patel, Pratik; Locsin, Miguel; Xiang, Yijin; Lu, Lydia; Fernandez, Alfred; and Jaggi, Preeti

Presenting Author: Pratik Patel, MD

Background: Evaluation for bacterial bloodstream infections (BSIs) is often associated with prescribing empiric antibiotics while awaiting blood culture results, typically 48 hours. We examined characteristics associated with positive cultures treated as BSI vs contaminant in children and BSIs associated with early (< 24 hours) time-to-positivity (TTP).

Methods: In a retrospective study of children (≤ 21 years) at our pediatric healthcare system, we abstracted demographic, clinical, and blood culture data from the electronic medical record for all initial positive bacterial blood cultures from March 2021 to June 2022. We excluded fungi and cultures collected within 14 days of a previous positive. TTP was calculated from time/date of collection to Gram stain report. Host status was categorized as previously healthy, immunocompromised (IC), and chronic condition/s (CC). A BSI was defined as a positive culture treated as bacteremia. BSI cultures were categorized as Gram-positive definite (GPD) pathogens, other Gram-positive (OGP), Gram-negative (GN), or polymicrobial (PM). Characteristics associated with prolonged TTP for BSIs were identified using mixed-effects logistic regression.

Results: There were 1068 positive cultures identified in 896 patients, with 756 (71%) cultures treated as BSIs and 702 of those (93%) positive in < 36 hours. Positive cultures drawn with adequate blood volume, in the setting of fever, from IC and CC children, and positive within 24 hours were significantly more likely to be treated as BSIs (all p< 0.05). The most common BSI was a GN pathogen (34.3%). On multivariate analysis, cultures collected from patients with fever, more than 1 positive culture, and positive with GPD, GN, and PM pathogens were associated with early TTP (p< 0.01).

Conclusions: We found that 93% of clinically significant BSIs in children were identified by 36 hours with BSIs with pathogenic organisms (GPD, GN, PM) associated with TTP < 24 hours. Reassessment of the need for empiric antibiotics after 24-36 hours should be considered.
GLP-1 Receptor Agonists - A Potential New Medication for Pediatric Non-Alcoholic Fatty Liver Disease

Authors: Choi, Erika; Ramirez Tovar, Ana; Arora, Shruti; Soler Rodriguez, Delys; Fadoju, Doris; He, Zhulin; and Vos, Miriam

Presenting Author: Ana Ramirez, MD

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children in the US, rising alongside an increase in obesity and type 2 diabetes (T2DM). Untreated, NAFLD leads to end-stage liver disease and is one of the leading causes of liver transplantation in adults. GLP-1 receptor agonists (GLP-1 RA) in adult populations with T2DM have been shown to improve non-alcoholic steatohepatitis through improvement in insulin resistance, the common mechanism behind the development of NAFLD and T2DM. Currently, no medications are available for managing NAFLD or obesity in the pediatric population. We assess the effects of GLP-1 RA on markers of NAFLD and insulin resistance to address the critical, unmet need for a pharmacotherapeutic agent for pediatric NAFLD.

Design/Methods: Fifteen patients from a T2DM clinic were identified and fulfilled the following inclusion criteria: diagnosed with pre-diabetes or T2DM, prescribed a GLP-1 RA in the prior 12 months, evidence of NAFLD indicated by elevation of alanine aminotransferase (ALT) to twice the upper limit of normal (>50 for males, >44 for females). Six patients were excluded due to either never starting the medication, documentation of inconsistent use, or not having a follow-up ALT. Nine patients were included. The average change between baseline and the first measurement after starting a GLP-1 RA was calculated for ALT, A1c, and BMI.

Results: Participants’ ages ranged from 14 to 17. ALT decreased by an average of 60% or 98 points within an average of 109 days. A1c decreased by an average of 2.2 points within an average of 114 days. BMI decreased by an average of 5% or 2.4 points within an average of 105 days. The most dramatic effect was seen within the first 180 days of starting the GLP-1 RA.

Conclusions: This case series highlights the potential use of GLP-1 RA in the management of pediatric NAFLD. There was a steeper reduction of ALT and A1c compared to BMI, suggesting that improvement in NAFLD may be independent of weight loss. More randomized, robust, placebo-controlled studies are needed to evaluate the effects of GLP-1 RA.
Caregiver Measures of Stress in Recollection of Childhood Seizure Events

Authors: O’Banion, David; Ray, Caroline; and Diaz, Ana

Presenting Author: Caroline Ray, MD

Background: Everyone can recall seeing their first seizure. Most patients having a seizure do not remember the event but can develop chronic psychological sequelae. Now, consider the caregiver who witnesses their child seizing. Little is known about the impact of a child’s serious health events on the mental and physical health of caregivers. In epilepsy, it is possible that a single seizure event is traumatic enough to induce PTSD symptoms in a caregiver; therefore, we would expect health implications for the caregiver and repercussions on the family and child from that caregiver suffering from PTSD.

Methods: During the clinical history in the routine visit in child neurology clinic, we will collect measures of autonomic responsivity in the caregivers of children who have had a seizure-like event. For the first 2 minutes, a quiet baseline recording is obtained followed by a recording during the caregiver’s recollection of the seizure event. Data points from recordings will then be analyzed and compared to a control group of non-seizure presentations. We will have 30 participants in each group for a total number of 60 participants. We will then conduct a follow up interview by phone using a standard PTSD symptom scale to assess for the development of PTSD symptoms remote from the event.

Results: Project is currently enrolling with preliminary results available for consideration of statistical methods. Skin conductance values will be compared with maximum values minus average baseline value reporting the caregiver’s response and in similar studies have found to be predictive of PTSD development.

Conclusions: Conclusions to report the likelihood of PTSD development in caregivers that witness the event. Aim is to encourage early intervention for these caregivers. Trauma informed care practices may need to be considered in routine epilepsy clinics.
A group coaching pilot to study the feasibility and impact of cognitive-emotional skills in pediatric cardiology fellows

Authors: Rodriguez, Zahidee; Sadler, Anne; and Border, William

Presenting Author: Zahidee Rodriguez, MD

Project objective/background: Physician trainees experience unique stressors that contribute to burnout, including increasing responsibility, accelerated mastery of knowledge and skills, financial stress, comparison, and the perception of powerlessness. Various wellness initiatives have mixed results, however, there is no standardized curriculum or intervention. Coaching has been reported to positively impact trainee wellness by shifting perspective and meaning-making through examining values, beliefs, and attitudes in the face of challenges. We piloted a group coaching program to study the feasibility and preliminary impact on pediatric cardiology fellow wellness.

Methods/approach: We conducted a prospective observational study of 15 voluntary, one-hour workshops during curricular time facilitated by a physician-coach from August to December 2022 for 15 pediatric cardiology fellows. Each workshop had one central theme and utilized facilitated discussion, self-reflection prompts, and live coaching. Workshops were recorded when appropriate and self-study modules were available. The first session was an introduction and the remaining topics covered were story versus fact, feelings, processing pain, intentional models and ladder thoughts, ambiguity, emotional adulthood, hidden expectations, boundaries and perfectionism, imposter syndrome, self-worth, self-confidence, arrival fallacy, purpose and agency to overcome moral injury, and becoming future you. Pre- and post-surveys were optional and included: Neff’s Self-Compassion Scale – Short Form (SCS-SF), Perceived Stress Scale (PSS), nonproprietary single-item substitute for Maslach Burnout Inventory Emotional Exhaustion (MBI:EE) subscale and Clance Imposter Phenomenon Scale (CIPS). Unpaired t-test analysis with 95% confidence interval (CI) assessed the program’s effect on these metrics.

Results: 12 of 15 pediatric cardiology fellows completed a post-survey. Fellows engaged the most with live session compared to recorded or self-study participation options (Fig 1). Perceived stress and emotional exhaustion trended down while self-compassion trended up, although not statistically significant (Table 1). Imposter syndrome significantly increased mid-year (CI [-41.5, -23.3], Table 1). Feedback about the emotional impact was largely positive, with fellows reporting they are better equipped to process emotions (Fig 2).

Conclusion: Pediatric cardiology fellows self-reported community and normalization of their training experience, and learned self-reflection, self-compassion, and processing emotion through a feasible group cognitive-emotional coaching pilot that occurred during an established curricular training period.
Professional Development Curriculum to Promote Well-being Amongst Pediatric Cardiology Fellows

Authors: Rodriguez, Zahidee; Sadler, Anne; and Border, William

Presenting Author: Zahidee Rodriguez, MD

Project objective/background: Trainees have unique stressors like increasing responsibilities, leadership, career transitioning, ambiguity, and the logistics of navigating a career in medicine, both emotionally and operationally. Professional development can enhance competence, and effectiveness, thus alleviating some elements of burn-out (emotional exhaustion, depersonalization, and personal efficacy). Burnout can further be examined by domains of workplace culture (workload, control, reward, community, fairness, values). We pose a framework that intersects workplace culture with concepts of a healing culture, to yield a novel design for a professional development curriculum to promote wellbeing.

Methods/approach: Based on focus groups of pediatric cardiology fellows and early career faculty, 13 didactics were offered during normal curricular hours for pediatric cardiology fellows to voluntarily participate in between May, 2021 and January, 2022. Topics were chosen to be specific to the three pillars of burnout. Depersonalization was broadened to include identity to career. Healing culture concepts gave a framework to address workplace culture domains (Table 1). Following completion of the program, fellows volunteered to participate in a standardized interview until reaching saturation.

Preliminary Results: Of 19 pediatric cardiology fellows, 15 completed interviews. Figure 1 is a Sankey diagram of which didactics were most impactful. Demystifying career transition and identity was the most impactful theme among all fellows (career depersonalization, n=25). PGY5 and PGY6 fellows reported some impact from topics surrounding emotional exhaustion (n=10), and the least from accomplishment (n=5). However, PGY4 fellows found them equally impactful. Within each of the three themes, the impact of specific didactics varied (Figure 1).

Conclusion: The stress profile of pediatric cardiology fellows may change during training. Professional development activities can be curated to target specific burnout themes and overall foster wellbeing.
Adherence to Resuscitation Ergonomics Amidst High Rates of PICU Staff Turnover

Authors: Sehgal, Ila; Colman, Nora; McGough, Jennifer; Edwards, Nicole; and Hebbar, Kiran

Presenting Author: Ila Sehgal, DO

Background: Each year in the United States there are more than 6000 pediatric in-hospital cardiac arrests (IHCA) with low survival rates. The PICU, where the majority of IHCA occur, is an inherently chaotic environment where patient acuity and stakes are high. Pediatric patients experience acute and unexpected clinical deterioration making the ICU environment dynamic and complex.

Resuscitation ergonomics improves patient outcomes by optimizing the interaction of the environment with human factors driven behaviors. In 2021, the PICU implemented a simulation project to understand barriers to optimal resuscitation ergonomics. Inefficient supply allocation and team member positioning were identified as key areas impacting communication, effective CPR, and CPR orchestration. These elements to improve resuscitation ergonomics were then embedded into a simulation-based team training education initiative.

Methods: Recently, we experienced high rates of staff turnover making it challenging to ensure that the ad hoc team that responds to IHCA is made up of providers with prior resuscitation training. No data describes the impact of staff demographics on resuscitation ergonomics and optimal CPR performance. This study purpose is to determine the optimal number of team-trained staff on a resuscitation team needed to maintain high-quality resuscitation ergonomics during IHCA in our PICU. Demographic data and a Resuscitation Ergonomics Compliance Assessment or best practices checklist is filled out after each arrest. We hypothesized that 25% of trained clinical providers need to be present during an IHCA to maintain all resuscitation ergonomics.

Results: Total compliance scores were calculated by summing the 15 measure items from the best practices checklist for each cardiac arrest event and summarized using means and standard deviations. Pairwise evaluations for differences in mean total compliance scores between simulation categories were evaluated using Cohen’s d differences. None of the three simulation trained groups were able to maintain all 15 simulation ergonomic components.

Conclusions: A higher percentage of team trained Staff correlated with a higher compliance of resuscitation ergonomics best practices during IHCA. Subgroup analysis for each item on the checklist is underway. Data collected from this study will serve as building blocks as we roll out a system wide initiative introducing the CPR coach into our resuscitation team.
Investigating Rurality and its Association with Initiation of Childhood Cancer Survivor Care

Authors: Strange, Liberty; Lewis, Rebecca; Ji, Xu; Mertens, Ann; and Effinger, Karen

Presenting Author: Liberty Strange, MD, MPH

Background: Research has shown that childhood cancer survivors (CCS) are at increased risk of chronic health conditions due to their treatment. Many large pediatric cancer treatment centers have survivor programs that help screen for these chronic health conditions. These centers are typically located in metropolitan areas and serve catchment areas often including rural populations. While studies have evaluated distance from survivor clinic as a variable leading to disparate survivor care, rurality has been understudied. Our primary objective is to examine differences in survivor clinic initiation and follow up between rural and urban CCS.

Methods: This is a retrospective analysis of patients followed in the Aflac Cancer and Blood Disorders Center who were diagnosed with cancer at <21 years, completed therapy between 2009-2017, and were alive at 2 years from completion of therapy. Sex, race, ethnicity, diagnosis, treatment, zip code, insurance, and date of first survivor clinic visit were abstracted from the medical record. Patient zip codes at diagnosis were converted to Rural-Urban Commuting Area (RUCA) codes with rural codes defined as those in which <30% of workers commute to an urbanized area. Chi-square and Kaplan-Meier analysis were performed.

Results: Within our cohort of 1182 eligible patients (52% male, 49% non-Hispanic White, 51% leukemia/lymphoma survivor), 10.4% were classified as living in a rural area. Compared with 71.0% of urban CCS, 62.6% of rural CCS attended an initial survivor clinic visit [X² = 3.33, p-value = 0.07]. For CCS who attended an initial survivor clinic visit, the median time to clinic visit was 30.6 months after completion of therapy for rural CCS and 31.6 months for urban CCS [HR 1.11 (0.88,1.41), p=0.4].

Conclusions: Less rural survivors had an initial survivor clinic visit compared with urban survivors although this did not meet statistical significance. Survivors were seen for their initial survivor clinic visit at a median of 31.4 months from the completion of therapy with no difference by rurality. We anticipate that additional subgroup analysis, covariate analysis, and analysis of subsequent survivor clinic follow up visits may uncover a population of survivors that could benefit from future interventions to aid in improved survivor care.
A Comparison of Postoperative Analgesia for Nuss Procedure with Cryoablation Patients Receiving Single Shot Nerve Blocks (SSRNB) with and without Patient Controlled Intravenous Analgesia (PCIA)

Authors: Tolly, Renee’; Bohling, Amy; Bartels, Ashley; Dalby, Ally; Liu, Katie; Alalade, Emmanuel; and Bansal, Vipin

Presenting Author: Renee’ Tolly, MD, MHA

Background: The post-operative management of Nuss procedure for treatment of pectus excavatum is challenging due to inadequate pain control leading to prolonged opioid use, increased length of stay and higher readmission rates. Peripheral nerve blocks has surpassed neuraxial analgesia for post-operative pain management for Nuss procedures due to introduction of cryoablation. Our study aimed to investigate if SSRNB would be non-inferior to SSNRB with PCIA for pain management in this patient population.

Methods: A retrospective review was conducted of patients undergoing Nuss procedure with cryoablation at Children’s Healthcare of Atlanta-Scottish Rite in Atlanta, GA from December 1, 2015 to May 30, 2022. The primary outcome variable was total OMEs at 6, 12, 24, and 48 hours post operative. For secondary outcomes, we expanded the variables to include numeric pain scores at 6, 12, 24, and 48 hours post-operative, post-operative day (POD) to ambulation, post operative nausea and vomiting (PONV), POD to oral intake, LOS, medication administrated during the operative case and in the recovery room, type of regional, block medication and additives, and complications. Descriptive analyses were performed to calculate median, Inter Quartile Range (IQR), frequency, and percentage. To compare the differences between with and without PCIA, Fisher’s exact tests were used for categorical variables, and Wilcoxon sum rank tests were used for continuous variables. A p-value < 0.05 was considered statistically significant.

Results: 6/34 patients (17.6%) received SSRNB with PCIA while 28/34 (82.4%) received SSRNB only. SSRNB with PCIA patients were administered statistically significantly more opioids at the 6, 12, 24, and 48 hours post operative (p < 0.001). There is no statistically significant difference in pain scores, POD to oral intake, PONV, medication administration during the operative case and recovery room. SSRNB only patients had a statistically significant decrease LOS (median LOS = 2 days, p value < 0.002) and were able to ambulate earlier (p = 0.006). The median amount of dexmedetomidine in a SSRNB was statistically significant (p=0.013) with patients receiving a PCIA.

Conclusion: In patients undergoing Nuss Procedures, SSRNB only patients had statistically significant decreased OMEs administered and decreases in time to ambulation and LOS.
Outpatient Utilization of the RAM Cannula for Nasal Noninvasive Ventilation in Children

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Background: The RAM cannula consists of nasal prongs that can be used to deliver oxygen, continuous positive airway pressure (CPAP), and bilevel positive airway pressure (BPAP). Although the utility of RAM cannula to effectively provide non-invasive ventilation (NIV) has been studied in pediatric inpatients, there is limited data on the use of the RAM cannula to deliver NIV in the outpatient setting. The aims of our study were to describe the clinical characteristics and outcomes of children managed with outpatient nasal NIV via RAM cannula.

Methods: We performed a retrospective review of children aged 0-18 years treated with NIV via RAM cannula at our institution from 2010-2022. Subjects were included if they used RAM cannula in the outpatient setting or in the inpatient setting with the goal of home use. The analyzed data included age, underlying diagnosis, indication, duration of use, complication, and outcomes.

Results: We identified 20 children who utilized nasal NIV via RAM cannula in the outpatient setting. We identified an additional 6 patients for which NIV via the RAM cannula was attempted for home use, however they could not be discharged on this support. The median (IQR) age was 5.8 months (2.4-9.9). The most common diagnoses were bronchopulmonary dysplasia (20%), Trisomy 18 (30%), and restrictive lung disease (25%). Indications for respiratory support were hypercapnia (n=10), obstructive sleep apnea (OSA, n=9), and sleep-related hypoventilation (n=3). RAM cannula was utilized for inability to tolerate conventional interfaces (n=16), tracheostomy avoidance (n=11), relief of dyspnea (n=12), and patient comfort (n=2). At the end of the study, 11 patients were still receiving NIV via the RAM cannula at home. For the other 9 patients, 3 (33%) had switched to a conventional PAP mask, 3 (33%) had improvements in their lung disease and no longer received PAP, and 3 (33%) died. There were no complications associated with the use of RAM cannula.

Conclusion: Our study highlights the safety and utility of nasal NIV via RAM cannula in children with a variety of diagnoses in the outpatient setting until achievement of clinical improvement, tolerance of conventional interfaces, and to avoid tracheostomy.