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Abstract Book

Oral & Poster

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C1. National Estimates of Substance Use, Substance Use Disorders, and Treatment among Adolescent and Young Adult Cancer Survivors

Authors: Ji, Xu; Cummings, Janet; Mertens, Ann; Wen, Hefei; and Effinger, Karen E.

Presenting Author:	**Xu Ji, PhD**; xu.ji@emory.edu
Type:	Clinical - Oral

BACKGROUND: Adolescent and young adult (AYA) survivors of cancer are at an elevated risk of morbidity and early mortality due to cancer treatment. Substance use can exacerbate survivors' vulnerabilities and place them at further risk for adverse health outcomes. This study provides national estimates of the prevalence of substance use and misuse, substance abuse or dependence (i.e., substance use disorders [SUD]), and receipt of treatment for SUD among AYA cancer survivors.

METHODS: We used the 2015-2018 National Survey of Drug Use and Health data to identify a nationally-representative AYA sample (aged 12-34 years). Outcomes included past-year alcohol use, marijuana use, other illicit drug use, misuse of any prescription psychotherapeutic drugs (including opioid analgesics, stimulants, sedatives, or tranquilizers), and misuse of prescription opioid analgesics. Outcomes also assessed past-year SUD. Among those with SUD, we evaluated past-year receipt of SUD treatment. Multiple logistic regressions were estimated to compare outcomes between 846 AYAs who reported a cancer history and 142,870 AYAs who did not, adjusting for sociodemographic and need-related characteristics.

RESULTS: In bivariate analyses, AYAs with a cancer history were more likely than noncancer peers to use alcohol (78.6% vs. 63.4%; $p<0.001$) and illicit drugs other than marijuana (11.2% vs. 7.8%; $p=0.02$), misuse any prescription psychotherapeutic drugs (16.9% vs. 10.6%; $p<0.001$) and prescription opioid analgesics (12.0% vs. 5.9%; $p<0.001$), and have an illicit drug (other than marijuana) SUD (3.7% vs. 1.3%; $p<0.01$) in the past year. In regression analyses, differences in past-year misuse of any prescription psychotherapeutic drug and prescription opioid analgesics persisted ($p=0.02$, $p<0.01$, respectively). Among AYAs with SUD, those with a cancer history were more likely than noncancer peers to receive SUD treatment (21.0% vs 8.1%; $p=0.01$) in the past year; this difference persisted in regression analyses ($p=0.03$).

CONCLUSIONS: AYAs with a cancer history had an elevated risk for misusing prescription psychotherapeutic medications, which was driven by misuse of prescription opioids; yet, only one in five AYAs with cancer history and SUD received treatment. Our findings underscore the need for future interventions designed to reduce substance use and misuse and improve access to SUD treatment in AYA cancer survivors.

Oral Presentations: C1 - C4 (In Order by Presentation)

C2. The Adverse Childhood Experiences of Children Seen in Pediatric Emergency Departments: Common and Comparable to National and Local Data

Authors: Okeson, Karli; Reid, Carmen; Mashayekh, Summer; Moran, Timothy; and Agarwal, Maneesha

Presenting Author:	**Karli Okeson, DO**; Karli.okeson@emory.edu
Type:	Clinical - Oral

BACKGROUND: Adverse childhood experiences (ACEs) are traumatic exposures occurring prior to age eighteen and can cause negative health effects. Screening and intervention for ACEs is predominately performed in primary care settings. Patients at risk for ACEs who lack primary care and are dependent on pediatric emergency departments (PEDs) may be missed; thus, PEDs may be an important venue to address ACEs.

OBJECTIVES: Characterize the prevalence of ACEs among patients in two PEDs compared to national and state data and identify healthcare utilization patterns in patients with ACEs.

METHODS: In this cross-sectional observational study, caregivers completed a tablet-based survey regarding their children's ACEs using questions from the 2016 National Survey of Children's Health (NSCH) questionnaire. Caregivers were also asked about acceptance of ACEs screening in PEDs and healthcare utilization. Inclusion criteria were English-speaking caregivers of patients aged 0-17 years not requiring acute stabilization. Results were compared to national and state data from the NSCH questionnaire by calculating risk differences and 95% confidence intervals. ED utilization was evaluated using multinomial logistic regression.

RESULTS: Over 6 months, 1,611 patients were approached and 1,011 (62.7%) were enrolled; 27.9% (95% CI: 25.2-30.7) of children had experienced one ACE, which was statistically higher than national and state comparative data. Two or more ACEs were seen in 18.4% (95% CI: 16.1-20.9). Notably, 11.5% of children with ACEs had not seen a primary care provider (PCP) for routine health care in the prior 12 months and 8.5% had no PCP altogether. Increased ACEs were associated with a lower probability of PCP visits relative to PEDs visits for acute care (OR = 0.88, 95% CI: 0.77-0.98, p = .02). Most caregivers (84.4%) felt comfortable discussing ACEs and would use resources (90.4%) provided by the PED.

CONCLUSION: ACEs are common in PEDs patients at levels similar to state and national data. Additionally, children with higher ACEs are more likely not have a PCP and have more ED visits. Caregivers reported acceptance of PED-based ACEs screening and related trauma resources. Given PED utilization patterns in children with ACEs, PEDs may be an integral site to optimize ACEs-based care to vulnerable populations.

Oral Presentations: C1 - C4 (In Order by Presentation)

C3. Avoidant-Restrictive Food Intake Disorder (ARFID): A New Treatable Comorbidity Associated With Food Allergy?

Authors: Patrawala, Meera; Vickery, Brian; Proctor, Kaitlin; Stubbs, Kathryn; and Sharp, William

Presenting Author:	**Meera Patrawala, MD**; mmeht26@emory.edu
Type:	Clinical - Oral

RATIONALE: Food allergy (FA) management requires elimination diets, which should be limited to the medically-necessary allergens. Clinical experience suggests that many families struggle with diagnostic confusion, broad elimination diets, and dysfunctional feeding behavior. We sought to estimate the overall prevalence of avoidant/restrictive food intake disorder (ARFID), a treatable condition newly described in the DSM-V, in a tertiary care FA clinic.

METHODS: Research psychologists conducted semi-structured interviews and chart review with the guardians of 50 consecutive children presenting for FA evaluation in Allergy/Immunology clinic, and collected exploratory data regarding demographics, growth parameters, food allergies, description of eating habits and specific food groups consumed, family impact, and other comorbid medical conditions.

RESULTS: Parents of 53 children (mean 7.8 years, range 2 months-17 years; 68% male) with median 2.7 (range 1-10) suspected or actual FA were included. 86% described family impact from FA. 58% restricted intake of foods beyond the known/suspected allergens. 28% reported child-initiated self-restriction. Mean weight-for-age percentile was 52% (range 0.78-99.24%). 24 (45%) met criteria for at least one ARFID subtype.

CONCLUSIONS: More than half of families in this sample restrict diets beyond that which is required out of precaution and/or because their child is “picky,” though most have normal growth parameters. Up to 45% may have ARFID, a DSM-V diagnosis characterizing dysfunctional feeding behavior that is reversible with highly effective feeding therapy. Implementing multidisciplinary feeding interventions may reduce FA-associated distress, enable incorporation of safe foods, and support a nutritionally complete diet while maintaining appropriate allergen avoidance.

Oral Presentations: C1 - C4 (In Order by Presentation)

C4. Serology in Children With Multisystem Inflammatory Syndrome (MIS-C)

Authors: Rostad, Christina A.; Chahroudi, Ann; Mantus, Grace; Lapp, Stacey A.; Teherani, Mehgan; Macoy, Lisa; Rostad, Bradley S.; Milla, Sarah S.; Tarquinio, Keiko; Basu, Raj; Kao, Carol; Linam, W. Matthew; Zimmerman, Matthew G.; Shi, Pei-Yong; Menachery, Vineet D.; Oster, Matthew E.; Edupuganti, Sri; Anderson, Evan J.; Suthar, Mehul; Wrammert, Jens; and Jaggi, Preeti

Presenting Author:	**Christina Allen Rostad, MD**; christina.rostad@emory.edu
Type:	Clinical - Oral

BACKGROUND: Differentiating MIS-C from COVID-19 or Kawasaki Disease (KD) using clinical characteristics or standard laboratory tests is challenging. We aimed to determine whether there were differences in SARS-CoV-2 serologic responses in hospitalized children with MIS-C compared to children with COVID-19 or other conditions, including KD.

METHODS: From March 17, 2020 - May 20, 2020, we identified hospitalized children meeting the CDC case definition for MIS-C, children symptomatic with PCR-confirmed COVID-19, and children with other conditions, including KD. We tested available sera from these groups of children to measure SARS-CoV-2 spike (S) receptor binding domain (RBD) binding antibodies by ELISA and SARS-CoV-2 neutralizing antibodies by focus reduction neutralization assay (FRNT).

RESULTS: Demographic, clinical, laboratory, and serological features were compared between groups of children with MIS-C (n=11), COVID-19 without MIS-C (n=21), and KD (n=6). Compared to COVID-19 and KD, children with MIS-C were significantly more likely to develop respiratory failure (P=0.0029), to have cardiogenic shock requiring vasopressors (P<0.0001), and to require intensive care (P=0.0014). Children with MIS-C had significantly higher RBD endpoint titers (geometric mean titer [GMT] 7548; n=9) than children with COVID-19 (GMT 698, P<0.0001; n=8), hospitalized children with KD and other conditions (GMT 117, P<0.0001; n=6) and healthy pediatric controls (GMT 85, P<0.0001; n=9). RBD IgG endpoint titers correlated well with SARS-CoV-2 neutralization titers among the groups (R2=0.6604, P=0.0056).

CONCLUSIONS: Subjects with MIS-C demonstrated high titers of SARS-CoV-2 binding and neutralizing antibodies. Quantitative antibody measurements may have a role in the evaluation of children with MIS-C.

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C5. Achieving Wellness After Illness for Teens (AWAIT): An App-Based Hope Intervention

Authors: Effinger, Karen E.; Williamson Lewis, Rebecca; Getachew, Betelihem; Mitra, Kuheli; Potts, Jessica; Berg, Carla; and Mertens, Ann C.

Presenting Author:	**Karen Effinger, MD, MS**; karen.effinger@emory.edu
Type:	Clinical - Poster
Poster Available:	Yes - P1
Poster Session Zoom Room Link:	Email Dr. Effinger with any questions.

BACKGROUND: Childhood cancer survivors (CCS) are at increased risk of morbidity and mortality due to treatment-related chronic conditions, which may be augmented by an unhealthy lifestyle. Hope, a positive psychological concept focused on goal-directed thinking, has been associated with engaging in health-promoting behaviors. Achieving Wellness After Illness for Teens (AWAIT) is a behavioral intervention focused on increasing hope in order to improve quality of life (QoL) and health-promoting behaviors in adolescent CCS.

OBJECTIVES: To test the feasibility and acceptability of the AWAIT intervention.

METHODS: CCS aged 13-17 years and >3 months from therapy completion were randomized 2:1 to the AWAIT intervention or attention control stratified by diagnosis and time since therapy completion (<3 years or ≥3 years). Those randomized to the 8-week AWAIT intervention received access to weekly coaching calls to discuss progress towards patient-selected goals and a mobile app with weekly video modules, practice exercises and behavioral and mood tracking. Participants completed a modified Children's Hope Scale, PedsQL v4.0 and assessments of diet and physical activity at baseline and 2 months. Intervention acceptability was measured at the end of intervention. Differences between groups were assessed using Fisher's exact and Wilcoxon Rank Sum tests. Changes within the groups were evaluated using McNemar's and paired t-tests.

RESULTS: Overall, participants (n=48) were median age 15 years, 54% male and 42% leukemia/lymphoma CCS with no differences between the groups. Twenty participants (63%) completed intervention activities in at least 7 of 8 weeks (prior to COVID19 pandemic 20/28 [71%], after pandemic 0/4 [0%]). Currently 34 participants (71%; AWAIT 21/32 [66%], Control 13/16 [81%]) have completed the 2-month evaluation. Compared to baseline, there was no change in hope or QoL in either arm. AWAIT participants decreased hours of television (p=0.08) and other screen time (p=0.03). Most participants (86%) were satisfied with the intervention; 95% would recommend it to another CCS; 76% felt the information was relevant; and 90% felt the coaching calls were helpful.

CONCLUSIONS: Implementation of the AWAIT intervention is feasible and adolescent CCS found it acceptable. Future analyses will evaluate the ideal time from completion of therapy for AWAIT participation.

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C6. Cobalamin Deficiency in Children With Sickle Cell Disease: An Unanticipated Risk for Use of Nitrous Oxide Gas

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

Presenting Author:	**Rachel Krieger, DO**; rkriege@emory.edu
Type:	Clinical - Poster
Poster Session Zoom Room Link:	**Visit Krieger Zoom Room**

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

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

RESULTS: Ninety-four children with SCD and pain were enrolled. Median age was 13 years (Q1, Q3: 10, 16), 51% female, 68% Hb-SS, and 71% were on hydroxyurea (Table 1). Twenty-six percent (24/94) of patients demonstrated evidence of B12 deficiency, 25% of whom demonstrated a severe B12 deficiency (6/24). Another 7% (7/94) demonstrated possible deficiency. There were no statistically significant differences in age, gender, SCD genotype, hemoglobin levels, MCV or hydroxyurea use in those with and without B12 deficiency.

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

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C7. Cerebral Effect of Blood Transfusion in Children with Sickle Cell Disease

Authors: Lee, Seung Yup; Sathialingam, Eashani; Cowdrick, Kyle; and Buckley, Erin

Presenting Author:	**Seung Yup Lee, PhD**; seung.yup.lee@emory.edu
Type:	Clinical - Poster
Poster Session Zoom Room Link:	**Visit Lee Zoom Room**

Chronic blood transfusion is the current standard therapy to reduce the risk of primary and recurrent stroke in children with sickle cell disease (SCD). Despite the beneficial effects of transfusion, it poses a long-term burden to patients and their family due to significant risks. Thus, individualized monitoring of response to transfusion may help optimize the therapy and further mitigate risk of infarctions. In this study, we present low-cost, portable and non-invasive optical techniques that can quantify tissue-level cerebral blood flow (diffuse correlation spectroscopy, DCS) and oxygenation extraction fraction (frequency-domain near infrared spectroscopy, FDNIRS) and aim to determine if FDNIRS/DCS can detect changes in cerebral hemodynamic/metabolic status that occur in response to transfusion.

To date, we have recruited six children with SCD undergoing chronic transfusion therapy at CHOA. To assess the effects of transfusion, brief (< 5 min) FDNIRS/DCS measurements were made immediately prior to the start and end of transfusion. For each FDNIRS/DCS measurement, the optical sensor was manually held over patients' forehead to acquire blood flow index (BFI) and oxygen extraction fraction (OEF). Oxygen metabolism (CMRO2) was also calculated using BFI and OEF.

One patient data was excluded due to poor data quality. Of the remaining 5 patients, OEF, BFI and CMRO2 decreased after transfusion in 4 patients by a median of -30.0%, -6.4% and -10.2% , respectively. Compared to results of a previous MRI study that quantified both OEF and CBF response to transfusion in a similarly aged cohort, our FDNIRS-measured OEF decrease is comparable. However, the DCS-measured BFI decrease is more prominent than previously reported (30% vs. 9%). Moreover, CMRO2 decreases after transfusion while MRI study has reported no difference between before and after transfusion. The enhanced sensitivity of BFI to CBF in sickle cell disease was reported in our recent study and is likely attributed to the confounding influences of hematocrit.

Our results demonstrate that hybrid optical spectroscopies are sensitive to the expected decreases of CBF and OEF after transfusion. These data suggest that optical technique may be a promising bedside tool for real-time monitoring of therapeutic efficacy, which ultimately lead to personalized monitoring.

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C8. Quantifying the Difference in Risk of Adverse Events by Induction Treatment Regimen in Pediatric Acute Lymphoblastic Leukemia

Authors: West, Zachary; Castellino, Sharon; Monroe, Caitlin; Thomas, Amanda; McCracken, Courtney; and Miller, Tamara

Presenting Author:	**Tamara Miller, MD, MS**; tamara.miller@emory.edu
Type:	Clinical - Poster
Poster Available:	Yes - P2
Poster Session Zoom Room Link:	**Visit Miller Zoom Room**

BACKGROUND: Despite recent advancements, children receiving induction therapy for Acute Lymphoblastic Leukemia (ALL) still experience adverse events (AEs). This study compared induction morbidity by treatment regimen (3-drug vs. 4-drug).

METHODS: This retrospective cohort included patients with B- or T-cell ALL ages 1-21 years who received induction chemotherapy at Children's Healthcare of Atlanta between January 2010 and May 2018. Manual chart abstraction identified and graded 20 clinically-relevant AEs, readmissions, and ICU admissions. Outcomes were compared across induction regimens in multivariate analyses using Cox proportional hazard ratios (HR) with 95% confidence intervals (CI).

RESULTS: Among 486 eligible patients, 378 (77.8%) experienced at least one AE during induction. Rates were comparable between therapy groups. In adjusted analyses, The adjusted risks of sepsis (HR=2.16, 95% CI: 1.11, 4.19), hypoxia (HR 2.08, 95% CI: 1.03, 4.18), hyperbilirubinemia (HR 2.48, 95% CI: 1.07, 5.74), hyperglycemia (HR 2.65, 95% CI: 1.29, 5.42), thromboembolic event (HR 4.50, 95% CI: 1.30, 15.6), and hyponatremia (HR 7.88, 95% CI: 1.26, 49.4) were significantly higher in the 4-drug group. The risk of hypertension was higher in the 3-drug group (HR 0.67, 95% CI: 0.46, 0.96). There were no differences in hospital readmission or ICU admissions rates, but patients receiving 4-drug induction had greater total number of inpatient days (12 days, interquartile range (IQR) 5-20 vs. 4 days, IQR 3-8; $p<0.0001$).

CONCLUSIONS: Patients receiving 4-drug induction therapy were more likely to experience clinically significant AEs and had significantly longer hospitalizations. This information can inform supportive care practices during ALL induction and guide patients and families.

C9. Evaluation of Changes in Use of Methadone and Benzodiazepines Following Implementation of an Analgosedation Protocol in the NICU

Authors: Ocampo, Claudia; Hamrick, Shannon; and David, Kaitlin

Presenting Author:	**Claudia Ocampo, MD**; claudiaocampo90@yahoo.com
Type:	Clinical - Poster
Poster Available:	Yes - P3
Poster Session Zoom Room Link:	**Visit Ocampo Zoom Room**

Neonatal research has focused its attention on the potential long-term side effects of analgesics and sedatives on child development. New evidence points at adverse neurodevelopmental outcomes and brain injury caused by certain drugs such as benzodiazepines, ketamine and opioids. Notably, medications which act via the NMDA and GABA receptors have been shown in animal models to be implicated in affecting synapse formation as well as neuroapoptosis in developing brains, leading to worse neurocognitive outcomes later in life. This Quality Improvement project studies an analgesia/sedation protocol created with the purpose of decreasing the prevalence of neurotoxic agents used to manage pain and agitation in post-surgical patients in our NICU.

KEYWORDS: Analgosedation, NICU, dexmedetomidine, methadone, morphine, benzodiazepines

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C10. Investigating the Contribution of Unique Risk and Protective Factors Associated With Health-Related Quality of Life in Newly Diagnosed Pediatric Inflammatory Bowel Disease

Authors: Reed, Bonney; Shih, Sharon; Cushman, Grace; and Kugathasan, Subra

Presenting Author:	**Bonney Reed, PhD**; ebreed@emory.edu
Type:	Clinical - Poster
Poster Available:	Yes - P4
Poster Session Zoom Room Link:	**Visit Reed Zoom Room**

OBJECTIVE: Health-related quality of life (HRQOL) is typically examined from a deficit standpoint, meaning that little is known about factors associated with resilient outcomes in pediatric illness samples. The aim of the current study was to use a risk and resilience framework to investigate demographic, disease, and temperamental factors associated with child and parent-report of health-related quality of life in youth newly-diagnosed with inflammatory bowel disease (IBD).

METHODS: Participants included 54 caregivers and 56 youth ages 8 to 17 diagnosed with IBD who each completed ratings of the child's HRQOL using the IMPACT-III, a disease-specific measure. Parents rated their child's emotional reactivity, conceptualized as a risk factor, and adaptability, conceptualized as a protective factor. Disease symptoms were rated by youth, and physician global assessment of disease activity was obtained.

RESULTS: HRQOL was rated lower by children and their parents as self-reported disease symptoms and parent-rated emotional reactivity increased. Conversely, total HRQOL was higher for children with higher parent-ratings of adaptability. In multiple regression analyses, higher levels of adaptability along with male sex and lower child-reported disease symptoms were associated with higher child, $R2 = .73$, $F = 31.33$, $p < .001$, and parent-reported, $R2 = .43$, $F = 9.01$, $p < .001$, HRQOL.

CONCLUSIONS: Attention should be given to protective as well as risk factors associated with differential outcomes in HRQOL for youth newly-diagnosed with IBD. By focusing on protective factors, a strengths based approach may offer patients the opportunity to maximize HRQOL when facing a new IBD diagnosis.

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C11. The Role of Pre-Extubation Caffeine on Extubation Success in Preterm Infants

Authors: Salem, Anand; and Dryer, Rebecca; and Saroha, Vivek; and Patel, Ravi

Presenting Author:	**Anand Salem, DO**; asalem2@emory.edu
Type:	Clinical - Poster
Poster Session Zoom Room Link:	Visit Salem Zoom Room

BACKGROUND: In the neonatal intensive care unit, caffeine is commonly administered prior to planned extubation, and its use is associated with increased extubation success. Despite the broad use of caffeine, there is no established optimal time for its administration in the peri-extubation setting. This leads to some infants receiving caffeine immediately prior to planned extubation while others may receive caffeine 20 or more hours before extubation.

HYPOTHESIS: Infants who receive caffeine more proximally to extubation will demonstrate greater odds of extubation success compared to those that receive caffeine further from extubation.

METHODS: We conducted a retrospective observational cohort study at a single level III unit of very low birth weight infants born between August 2008 and July 2017. We evaluated the association between the timing of caffeine administration prior to extubation and extubation failure (defined as needing re-intubation within 1 week of extubation) using multivariable logistic regression. We specified timing of caffeine using tertiles and adjusted for confounding variables, including gestational age, weight at extubation, caffeine dose, and pH prior to extubation.

RESULTS: We evaluated 205 infants, with a mean gestational age of 26.8 weeks (SD 2.1) and a mean weight at extubation of 987 grams (SD 292). The median pre-extubation caffeine dose was 5.6 mg/kg (IQR 5.0-20.0 mg/kg). Extubation failure occurred in 67 infants (33%). There was no significant difference in extubation failure among caffeine groups, with an incidence of 32%, 37% and 29% in the 1st, 2nd and 3rd tertiles (with relation to caffeine timing prior to extubation) respectively. Gestational age at birth was the most significant factor associated with extubation failure ($P<0.001$).

CONCLUSION: Our study does not support the hypothesis that use of caffeine more proximal to extubation improves extubation success in very low birth weight infants.

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C12. Racial and Socioeconomic Disparities in Non-Accidental Trauma Screening of Infants Presenting With Simple Skull Fractures

Authors: Sooknarine, Hayley; and Richer, Edward

Presenting Author:	**Hayley Sooknarine, MD, FAAP**; Hsookna@emory.edu
Type:	Clinical - Poster
Poster Available:	Yes - P5
Poster Session Zoom Room Link:	**Visit Sooknarine Zoom Room**

AIMS: 1) Evaluate NAT screening practices of young infants presenting with apparently isolated skull fractures, and 2) to identify which infants have a high level of concern for NAT.

METHODS: This was a retrospective chart review. Inclusion criteria were patients under 6 months of age presenting to 2 metropolitan Pediatric trauma centers (academic and non-academic) with simple skull fractures between January 2014 and December 2016. Patients were excluded if there was high level of concern for NAT on presentation.

The study population was divided into 2 groups: infants undergoing skeletal surveys (SS) versus not. Data analysis was performed using $\chi 2$ and odds ratio (OR) calculations.

RESULTS: 157 infants were included. 64 infants (40.8%) underwent NAT screening with skeletal survey, and 1 of these surveys showed additional fractures (1.6% of those screened). The infant with the positive SS had no history to explain the fracture.

66% of black infants were screened, 31.4% of white infants were screened, and 25% of Asian/other infants were screened ($P<0.001$). The academic hospital screened 53% of presenting infants, while the non-academic hospital screened 33% ($P<0.001$). Over 2 times more black infants were screened at the academic hospital vs. 1.33 times more were screened at the non-academic hospital ($P = 0.05$). Conversely, only 26% of white infants were screened at the non-academic hospital compared to 41.4% at the academic hospital ($P<0.001$).

87% of black infants were publicly insured versus 47.7% of white infants and 79.2% of Asian/other infants. Of the publicly insured, 29/41 black infants (70.7%) were screened versus 20/41 white infants (48.8%). 4/19 (21%) Asian/other publicly insured patients were screened. The odds ratio for being screened if publicly insured was 4.517 (CI: 2.0993 to 9.7191, $P=0.0001$). Both hospitals saw lower numbers of privately insured patients; however, a smaller proportion of privately insured patients were surveyed at the non-academic hospital (15.8%) compared to the academic hospital (27.8%) ($P<0.0001$).

CONCLUSION: There are both racial and socioeconomic disparities in screening for this injury with low specificity but potential nonetheless for physical abuse. There is a need for appropriate protocols to reduce disparities for NAT screening of patients.

C13. Impact of Gut Microbiome Changes on Hematopoietic Stem Cell Transplantation Outcomes in Children

Authors: Teherani, Mehgan; Pratte, Zoe; Banksota, Samridhi; Gulick, Dalia; Djeddar, Naima; Horan, John Gibson, Gregory; and Qayed, Muna

Presenting Author:	**Mehgan Teherani, MD, FAAP**; mtehera@emory.edu
Type:	Clinical - Poster
Poster Available:	Yes - P6
Poster Session Zoom Room Link:	**Visit Teherani Zoom Room**

BACKGROUND: In adults undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), higher microbiome diversity results in reduced bloodstream infections (BSIs) and improved overall survival. We are investigating the use of rifaximin prophylaxis (ppx) in pediatric allo-HSCT in an ongoing trial to improve microbiome diversity and decrease BSI. Here we present our preliminary results.

METHODS: This ongoing study utilizes a prospective treatment group on rifaximin, with a retrospective control or children undergoing HCT for hematologic malignancy. The control cohort did not receive prophylactic antibiotics while the treatment cohort received rifaximin ppx through 28 days post-HSCT (D+28). BSIs caused by mucosal barrier injury and considered derived from the gut from blinded review were included. Most patients received piperacillin-tazobactam at the onset of fever. Timing of systemic antibiotics was categorized as none, early (before D0), and late (after D0). Primary outcome was death by D+180 post-HSCT. We performed 16S rRNA sequencing of weekly stool samples obtained at baseline, day 0 to D+28 and calculated the Shannon-index of diversity using QIIMEII.

RESULTS: We have enrolled 21 rifaximin patients with clinical outcomes. Twelve rifaximin patients and 61 controls were included in the microbiome analysis. Median age of the patients was 9 years (range 1-20). 41% Caucasian, 21% Hispanic, and 29% Black race.

We observed a significant drop in diversity for patients who received antibiotics early (Mean=2.7, SE=0.2) compared to late (M=3.7, SE=0.1), with highest diversity for patients who did not receive antibiotics (M=4.1, SE=0.4); p=0.0041). Death at D+180 was associated with a lower diversity at D+28 that patients who survived (M = 2.9 versus 1.73, p=0.04. 14% of the controls developed a BSI within the first 30 days post-HSCT. Early analysis of BSI in the rifaximin group shows only one gut BSI out of 21 patients.

CONCLUSIONS: We have shown a significant correlation between engraftment (D+28) microbiome diversity and 6-month overall survival. Early antibiotic exposure in our cohort was detrimental to microbiome diversity. Approaches to preserve microbiome diversity while preventing BSI are likely to improve transplant outcomes. Our ongoing trial using rifaximin ppx will provide data regarding this approach.

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C14. Use of a Novel Human Milk-Based Fortifier in Term Infants With Surgical Congenital Gastrointestinal Disorders Improves Growth at Discharge

Authors: Tweddell, Sarah; Barbian, Maria; and Karpen, Heidi

Presenting Author:	**Sarah Tweddell, MD, MS**; twedds@gmail.com
Type:	Clinical - Poster
Poster Available:	Yes - P7
Poster Session Zoom Room Link:	**Visit Tweddell Zoom Room**

BACKGROUND/PURPOSE: Human milk (HM) is the ideal source of nutrition for all infants, but alone is insufficient in protein and calories for infants with surgical congenital gastrointestinal disorders (S-CGD). Currently, the only option for term infants is fortification with formulas which have been associated with increased risk of necrotizing enterocolitis (NEC) in the preterm population and may contribute to the high rates of NEC in those infants with congenital gastrointestinal disorders. The purpose of this study is to demonstrate the safety and efficacy of a novel HMBF designed for term infants with S-CGD.

METHODS: This is a cohort of an ongoing multi-center, prospective, case-control, open-label comparative effectiveness trial of approximately 150 infants with qualifying surgical intestinal disorders (gastroschisis, omphalocele and intestinal atresias). Patients were fed an exclusive human milk diet of mother's own milk (MOM), and pasteurized donor human milk (PDHM, Prolacta Bioscience) fortified with a novel HMBF (PBCLN-002, Prolacta Bioscience) formulated for the term infant. The first 26 term infants enrolled in this trial were evaluated for safety and efficacy of PBCLN-002 compared to a cohort of patients fed diets containing MOM and formula. The retrospective cohort was matched based on diagnosis, sex, birthweight and gestational age.

RESULTS: Baseline characteristics were well matched between the groups. There were no adverse events in the PBCLN group and no significant effect on feeding tolerance, interruptions or maximum direct bilirubin. Infants receiving PBCLN-002 grew faster in terms of weight, length and comparative head circumference velocity; however, these were not statistically significant. Discharge weight and length were significantly higher for the PBCLN group as compared to the retrospective cohort. Adjusting for baseline using a basic analysis of covariance model, these differences remained significant for discharge weight ($p=0.02$) and discharge length ($p=0.003$).

CONCLUSIONS: In this small cohort of patients with surgical congenital gastrointestinal disorders, PBCLN-002 demonstrates safety and efficacy by increased growth at the time of discharge. The non-significant differences in daily weight gain and growth velocities between these two groups may reflect the small sample size and differences in parenteral nutrition support during the early phases of surgical recovery.

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C15. Parent Psychological Distress and Chronic Pain in Pediatric Sickle Cell Disease

Authors: Woodward, Kerri; Sil, Soumitri; Johnson, Yelena; Dampier, Carlton; and Cohen, Lindsey

Presenting Author:	**Kerri Woodward, PhD**; kerri.woodward@gmail.com
Type:	Clinical - Poster
Poster Available:	Yes - P8
Poster Session Zoom Room Link:	**Visit Woodward Zoom Room**

INTRODUCTION: Pediatric chronic pain can be associated with increased parenting stress and poor emotional and family functioning in pediatric sickle cell disease (SCD). Parenting stress has been associated with poorer child pain-related outcomes, including increased disability and reduced quality of life. This study aimed to investigate how parenting stress, parent emotional functioning, and family functioning 1) were associated with child outcomes and 2) differed based on the chronicity of pediatric SCD pain.

METHODS: Children and adolescents with SCD (n=77; Mage=14.24, SD=2.48) and their parents were recruited from comprehensive SCD clinics; 93.5% (n=72) completed study procedures. Youth were primarily African American (93.2%), female (55.8%), with hemoglobin type HbSS (72.7%); parents were mostly female (90.9%). Youth were classified as having chronic pain if they reported ≥15 pain days/month for ≥ 6 months (n=32, 41.6%). Youth with episodic pain reported <15 pain days/month (n=40, 51.9%). Parents completed the Pediatric Inventory for Parents, Center for Epidemiological Study – Depression Scale, Patient Health Questionnaire – Generalized Anxiety Disorder, and Family Assessment Device. Youth completed the Pediatric Quality of Life Inventory – SCD and Functional Disability Inventory.

RESULTS: Parenting stress frequency, parenting stress difficulty, and parent anxiety were significantly correlated with child functional disability and quality of life (all $p<0.05$). Parents of children with chronic SCD pain reported significantly more frequent parenting stress ($p<0.01$), more difficult parenting stress ($p<0.01$), and more symptoms of anxiety ($p=0.04$) than parents of youth with episodic SCD pain. Parent depressive symptoms and family functioning did not significantly differ by pain groups ($p>0.10$).

CONCLUSIONS: Results indicate that pediatric pain chronicity in sickle cell disease is related to increased parenting stress, and increased parenting stress and anxiety are associated with worse child outcomes. Future studies are needed to evaluate potential parent-focused interventions for managing stress and anxiety in the context of chronic pediatric pain.

Poster Presentations: C5 - C16 (In Order by Presenting Author Last Name)

C16. Development and Validation of the HIV Adolescent Readiness for Transition Scale (HARTS) in South Africa

Authors: Zanoni, Brian; Archary, Moherndran; Sibaya, Thobekile; Musinguzi, Nicholas; and Haberer, Jessica

Presenting Author:	**Brian Christopher Zanoni, MD, MPH**; bzanoni@emory.edu
Type:	Clinical - Poster
Poster Available:	Yes - P9
Poster Session Zoom Room Link:	**Visit Zanoni Zoom Room**

BACKGROUND: Adolescents living with perinatally-acquired HIV have low rates of viral suppression after transition to adult care. In South Africa, timing of transition to adult care is often arbitrary and occurs with little or no preparation.

MATERIALS AND METHODS: We iteratively created the HIV Adolescent Readiness for Transition Scale (HARTS) by conducting focus groups with healthcare providers (n=11), and adolescents (n=20 in 2 groups) before transition to adult care. We then administered the HARTS questionnaire to 131 adolescents to determine the psychometric properties of the questionnaire. Based on item response theory, we used generalized linear equation models with the overall score and with the individual domains. We correlated the responses to self-described transition readiness and age using liner regression. We then validated the scale by prospectively administering it to 199 different adolescents in a separate setting prior to their transition and measured viral suppression (viral load <200 copies/ml) one year after transitioning to adult clinic. We evaluated transition outcomes using multivariable logistic regression based on the continuous HARTS.

RESULTS: We identified 4 domains that were important to HIV transition readiness: disclosure, health navigation, self-advocacy, and health literacy. Fifteen of the sixteen questions were significantly associated with their domain and overall HARTS score. Positive correlations with self-described transition readiness were significant with the overall HARTS score ($p<0.0004$) and domains of health navigation ($p=0.028$), self-advocacy ($p=0.0014$), and health literacy ($p=0.0023$). Scores and domains increased with age but not significantly. In the prospective validation, the total 15 question HARTS score ranged from 2 to 56. For participants receiving first-line antiretroviral therapy, each 10-point increase in HARTS score was associated with 0.53 odds of viral failure ($p=0.001$; 95% CI 0.36 – 0.77) in our multivariable model adjusting for age at antiretroviral therapy initiation, sex, disclosure status, drug and alcohol use, peer support, and self-esteem. Age alone at time of transition was not significantly associated with viral suppression 12 months after transition to adult care.

CONCLUSION: The HARTS questionnaire is a validated scale that can be used to determine which adolescents may require additional interventions prior to transitioning to adult care to improve viral suppression.

Oral Presentations: B1 - B4 (In Order by Presentation)

B1. IRF5 Drives Potentiated IFN-beta and IL-12 Production by Dendritic Cells in Response to Combinatorial CpG- and MPLA- Delivery on Microparticles

Authors: Jhita, Navdeep; Toy, Randall; Pradhan, Pallab; Blanchard, Emmeline L.; Narang, Neha; Cortes, Gabriela; Keenum, M. Cole; Santangelo, Philip J.; Shayakhmetov, Dmitry M.; and Roy, Krishnendu

Presenting Author:	**Navdeep Jhita, MD**; njhita2@emory.edu
Type:	Basic - Oral

Vaccines have enabled control of many deadly infectious diseases. However, efficacy in host protective immune responses vary greatly. Adjuvants targeting Toll like and cytosolic receptors (TLR) can greatly enhance antigen-specific immunity. In this regard, pathogen-like micro-particles (PLP) co-loaded with TLR4 (MPLA) and/or TLR9 (CpG) ligands was used as a novel platform to deliver combinatorial adjuvants. We analyzed molecular signaling pathways activated in bone-marrow-derived dendritic cells (BMDCs) upon stimulation with PLPs, specifically leading to IFNb and IL-12p70 production. Stimulation of BMDCs with PLPs containing only MPLA or CpG resulted in low-level or no IFN-b and IL-12p70 release. However, stimulation of BMDCs with PLPs containing both MPLA and CpG triggered production of very high amounts of IFN-b and IL-12p70. Analysis of signaling pathways responsible for these results demonstrated IFN-b and IL12-p70 production in BMDCs harvested from WT, IRF3-KO, and IRF7-KO mice, but not from IRF5-KO animals. Exploration of IRF5 phosphorylation kinetics showed MPLA-loaded PLP transiently stimulated IRF5 (0.5 to 2 hours post stimulation) with dual adjuvant PLPs displaying a greatly prolonged p-IRF5, detectable even at 4 hours post stimulation. We are currently analyzing molecular regulators of p-IRF5 stability and kinetics of its translocation to the nucleus. Our study reveals an unexpected role for IRF5 in driving a highly potentiated production of immune-stimulatory cytokines from dendritic cells in response to dual MPLA and CpG adjuvants. Additionally, our findings may help develop novel vaccines with improved efficacy when combined with PLPs loaded with MPLA and CpG adjuvants that activate IRF5-dependent cytokine production.

Oral Presentations: B1 - B4 (In Order by Presentation)

B2. Reservoir Composition in a Rhesus Macaque Model of Pediatric HIV-1 Infection: Insights Into Targets for Remission Strategies

Authors: Obregon-Perko, Veronica; Mensah, Gloria; Bricker, Katherine; Berendam, Stella; Liang, Shan; Vanderford, Thomas; Kumar, Mithra; Fray, Emily; Silicano, Robert F.; Bar, Katharine; Shaw, George; Silvestri, Guido; Fouda, Genevieve; Permar, Sallie; and Chahroudi, Ann

Presenting Author:	**Veronica Obregon-Perko, PhD**; vobrego@emory.edu
Type:	Basic - Oral

Mother-to-child transmission of HIV-1 continues to contribute to new cases of infection in children, with breastfeeding being a major route. Very early initiation of antiretroviral therapy (ART) can impede establishment of the long-lived reservoir, but the window of opportunity is narrow and difficult to reach in resource-limited settings, creating a critical need for alternative cure strategies. Relative to studies in adults, little is known about the composition of the viral reservoir in the setting of peripartum transmission, which could differ from adults and require specific interventions. Here, we used orally simian/human immunodeficiency virus (SHIV)-infected ART-treated infant macaques as a model of pediatric HIV-1 infection to characterize cellular and anatomic reservoirs. SHIV-infected infant macaques showed sustained high-level plasma viremia and preservation of peripheral CD4 T cell frequencies during acute infection. Env-specific binding antibodies elevated concomitantly with plasma viral loads, but gradually declined after ART initiation. We tracked viral persistence through SHIV DNA/RNA in blood and tissue CD4 T cells. Although blood, lymphoid, and colorectal sites had similar SHIV DNA after a year on ART, SHIV RNA expression was significantly elevated in colorectal CD4 T cells, suggesting this is an active site of low-level transcription under suppressive ART. Virus in blood and spleen was confirmed to be replication-competent by viral outgrowth assay; levels in the spleen more closely modeled plasma viral burden prior to ART. Memory CD4 T cells are generally regarded as the major source of latent virus, but their low frequency during infancy raises the possibility of contribution from other cell types in pediatric infection. Indeed, SHIV DNA was detected in naïve cells at a comparable frequency to memory cells in infant macaques. Furthermore, proviral DNA within naïve cells was largely intact, rather than defective.

Considering their higher abundance in the infant CD4 T cell pool, naïve cells could represent a sizeable source of latent virus to be considered for cure efforts. In summary, we have used a pre-clinical macaque model of pediatric HIV-1 infection to evaluate reservoir composition and highlight distinctions from adults. Our use of a chimeric SHIV will also allow investigation of HIV envelope-targeting remission strategies.

Oral Presentations: B1 - B4 (In Order by Presentation)

B3. Discovery of Specific Ribosomal Proteins as Investigational Therapeutic Targets for Cystic Fibrosis

Authors: Oliver, Kathryn E.; Rauscher, Robert; Bampi, Giovana B.; Mao, Yiyang; Rab, Andras; Hong, Jeong S.; Hartman IV, John L.; Ignatova, Zoya; and Sorscher, Eric J.

Presenting Author:	**Kathryn Oliver, PhD**; kolive3@emory.edu
Type:	Basic - Oral

Cystic fibrosis (CF) is an autosomal recessive disorder associated with >1,600 mutations in the CF transmembrane conductance regulator (CFTR), a chloride and bicarbonate channel expressed at the apical surface of secretory epithelia. Deletion of phenylalanine 508 (F508del) is the most prevalent variant, conferring a protein folding abnormality with subsequent endoplasmic reticulum retention and proteasomal degradation, resulting in severe damage to exocrine tissues (e.g. lung, pancreas, intestine). CFTR variants have been traditionally grouped into six mechanistic classes based on molecular defects in synthesis (W1282X), maturational processing (F508del, G85E, P67L), gating (G551D), conductance through a malformed ion pore (R334W), steady-state surface protein (A455E), and plasma membrane turnover (N287Y). We previously applied an innovative approach (genome-wide yeast phenomics) to identify modifier genes that rescue mutant CFTR, revealing several robust 'hits' that cluster in distinct structural regions of the ribosome – most notably ribosomal protein L12 (RPL12/uL11). Using Fischer rat thyroid (FRT), CF human bronchial epithelia (CFBE), and primary human airway epithelia, we show that siRNA-dependent suppression of RPL12 results in significant enhancement of F508del-CFTR protein stability, maturation efficiency, plasma membrane trafficking, open channel probability, and transepithelial ion transport. We also generated haplosufficient Rpl12+/- mice and found that wild-type CFTR expression is markedly enhanced in the pancreas, colon, and ileum. Through use of ribosome profiling and 'omics' technologies, we discovered that RPL12 depletion diminishes rates of translation initiation and elongation due to increased ribosomal A-site dwelling occupancy of GC-rich codons. Because RPL12 is located at the base of the 60S P stalk and resides within the GTPase-associated center, we investigated whether RPL12 silencing might improve other CFTR variants exhibiting defects in folding (G85E, P67L), conductance (A455E), or premature termination (W1282X). Biochemical (western blot) and functional (short-circuit current) analyses established that RPL12 knockdown partially rescued three of the four variants tested, the most striking of which was W1282X-CFTR. The P stalk, therefore, appears to play a crucial role during mutant CFTR folding, stability, and/or translational fidelity. Taken together, these findings suggest RPL12 silencing may represent a novel therapeutic strategy for addressing multiple CFTR defects, particularly rare variants that remain without an effective clinical intervention.

Oral Presentations: B1 - B4 (In Order by Presentation)

B4. Arginine Dysregulation Correlates With Cardiac Remodeling in Mice With Chronic Kidney Disease

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

Presenting Author:	**Loretta Reyes, MD**; loretta.reyes@emory.edu
Type:	Basic - Oral

BACKGROUND: Nitric Oxide (NO) is critical for cardiovascular homeostasis. Arginine, the sole nitrogen donor for NO synthesis, is the common substrate for NO synthase and arginase enzymes. Low global arginine bioavailability ratio (GABR), a potential endothelial dysfunction biomarker, has been associated with increased mortality in adults with heart failure. Arginine metabolism is dysregulated in chronic kidney disease (CKD) and is associated with changes in myocardial function. This study aimed to determine the relationship between metabolites/enzymes in arginine metabolic pathways with cardiovascular measures in CKD mice and investigate the effect of arginine supplementation.

METHODS: CKD established in male 129X1/SvJ mice via 5/6th nephrectomy. Plasma collected at 8- and 16-weeks post-surgery was analyzed for arginine, citrulline, ornithine, asymmetric dimethylarginine (ADMA) via LC-MS/MS; GABR=arginine/(ornithine+citrulline). Arginase activity was analyzed via colorimetric assay. Echocardiographic measures of left ventricular hypertrophy (LVH), diastolic dysfunction and ventricular strain were obtained at 8- and 16-weeks. In a separate experiment, CKD and control mice received chow supplemented with arginine or alanine (nitrogen control) for 12 weeks. Plasma and echocardiograms obtained at 8- and 12-weeks were examined as above. Blood pressure was measured noninvasively.

RESULTS: In CKD mice, low GABR correlated with decreasing E/A ratio (measure of diastolic dysfunction) [r=0.58; p=0.01] and increasing relative wall thickness (RWT) (measure of LVH) [r=-0.49, p=0.03]. Plasma arginase activity was significantly increased in CKD mice at 16-weeks [median (IQR) 10.5 (8.4-11.7)] compared to controls [5.5 (1.5-10.0); p</= 0.05] and to CKD mice at 8-weeks [7.0 (3.7-7.6); p=0.002]. Increased arginase activity correlated with impaired ventricular strain [r=-0.34; p=0.04]. ADMA was significantly increased in CKD mice at 16-weeks [7.2 (7.1-7.3)] compared to controls [7.1 (7.1-7.1); p=0.036] and correlated with lower E/A ratio [r=-0.34; p</=0.05]. After 12-weeks of supplementation, arginase activity was significantly lower in arginine supplemented CKD-mice compared to arginine supplemented normal mice [7.8 (3.1-8.5) vs 14.3 (10.0-15.6); p=0.004] and compared to alanine supplemented CKD-mice [18.8 (12.9-19.7); p=0.006]. In arginine supplemented CKD-mice, blood pressure was significantly lower at 12 weeks compared to 8 weeks; p=0.03.

CONCLUSIONS: In a mouse model of CKD, dysregulation in arginine metabolism correlates with myocardial dysfunction; hypertension is ameliorated with arginine supplementation.

Poster Presentations: B5 - B10 (In Order by Presenter Last Name)

B5. A Patient-Specific 3D Bioprinted Platform for *In Vitro* Disease Modeling and Treatment Planning in Pulmonary Vein Stenosis

Authors: Serpooshan, Vahid; Tomov, Martin L.; Jing, Bowen; Kumar, Akaash; Panoskaltsis, Nicki; Mantalaris, Athanasios; Slesnick, Timothy C.; Lindsey, Brooks; and Bauser-Heaton, Holly

Presenting Author:	**Holly Bauser-Heaton, MD, PhD**; basuerh@kidsheart.com
Type:	Basic - Poster
Poster Session Zoom Room Link:	**Visit Bauser-Heaton Zoom Room**

Pulmonary vein stenosis (PVS) is an acute pediatric cardiovascular disease that is always lethal if not treated early. 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. Thus, there is an unmet need to develop functional *in vitro* models of PVS that can serve as a platform to study clinical interventions. Patient-inspired 3D bioprinted tissue models provide a unique model to recapitulate and analyze the complex tissue microenvironment impacted by PVS. Here, we developed perfusable *in vitro* models of healthy and stenotic pulmonary vein by 3D reconstruction and bioprinting of patient CT data (Figure 1). Models were seeded with human endothelial (ECs) and smooth muscle cells (SMCs) to form a bilayer structure and perfused using a bioreactor to study cell response to stenotic geometry, and to the stent-based treatment. Flow hemodynamics through printed veins were quantified via CFD modeling, 4D MRI and 3D ultrasound imaging. Cell growth and endothelialization were analyzed. Our work demonstrates the feasibility of bioprinting various cardiovascular cells, to create perfusable, patient-specific vascular constructs that mimic complex *in vivo* geometries. Deeper understanding of EC-SMC crosstalk mechanisms in *in vitro* biomimetic models that incorporate tissue-like geometrical, chemical, and biomechanical ques could offer substantial insights for prevention and treatment of PVS, as well as other cardiovascular disease.

B6. Epigenetic and Transcriptional Dynamics in Acute and Chronic Human Myeloid Inflammation

Authors: Cammarata-Mouchtouris, Alexandre; Moncada, Diego; Giacalone, Vincent; Dobosh, Brian; Prahalad, Sampath; and Tirouvanziam, Rabindra

Presenting Author:	**Alexandre Cammarata-Mouchtouris, PhD**; acammar@emory.edu
Type:	Basic - Poster
Poster Session Zoom Room Link:	**Visit Cammarata-Mouchtouris Zoom Room**

RATIONALE: Beyond receptors and signaling proteins, genetic, epigenetic and transcriptional regulators are important in the unfolding of immuno-inflammatory responses. Epigenetic and transcriptional regulators in particular allow for rapid adaptation of responses in different types of cells and pathophysiological contexts. For example, our laboratory demonstrated in patients with Cystic Fibrosis (CF) that neutrophils recruited from blood into the airway lumen undergo reprogramming that causes them to promote a complex reorganization of the local tissue, in coordination with macrophages and epithelial cells. Such reprogramming of human neutrophils runs counter to the conventional assumption that the fate of myeloid cells is pre-programmed and establishes these fast-acting subsets as adaptable coordinators of tissue responses.

APPROACH: In ongoing studies, we are expanding on our past investigations in CF to characterize epigenetic and transcriptional dynamics in other neutrophil-dominated diseases of peripheral tissues including Juvenile Idiopathic Arthritis, and late stage COVID-19. Our pipeline combines the Cut & Run epigenetic assay method with shotgun Illumina sequencing and long-read Oxford Nanopore MinION sequencing to understand the regulatory landscape of neutrophils and monocytes recruited to diseased airways and joints.

RESULTS: Output from Cut & Run assays conducted in tissue neutrophils and macrophages in CF and JIA will be compared to illustrate the scope and specificity of epigenetic and transcriptional adaptations occurring over time in these fast-acting subsets. Rapid epigenetic changes can sometimes remain in effect even after the initial stimulus has waned, leading to transcriptional memory (a component of trained innate immunity). Therefore, the potential impact on long-term immune polarization / dysregulation will also be outlined.

CONCLUSIONS: A better understanding of dynamic adaptations in myeloid subsets associated with human peripheral inflammation using this and other approaches will help identify novel epigenetic and transcriptional targets for therapeutic modulation of various intractable diseases.

ACKNOWLEDGMENTS: CTID Pilot and Cystic Fibrosis Foundation.

B7. Complement-Mediated Acute Lung Injury in Sickle Cell Disease: Novel Mechanisms and Therapies

Authors: Chonat, Satheesh; Patel, Seema; Jeffers, Lauren; Cisneros de la rosa, Eduardo; Fields, Earl; Archer, David; Koval, Michael; Joiner, Clinton, and Stowell, Sean

Presenting Author:	**Satheesh Chonat, MD**; Satheesh.chonat@emory.edu
Type:	Basic - Poster
Poster Session Zoom Room Link:	**Visit Chonat Zoom Room**

Sickle cell disease (SCD) is a common and life-threatening autosomal recessive hematological disorder that affects millions worldwide. Amongst acute complications in SCD, acute chest syndrome (ACS) is a leading cause of hospitalization and the most common cause of death due to SCD. Unfortunately, supportive care remains the primary approach to alleviate these complications. This in part reflects an incomplete understanding of the pathophysiology and accompanying pharmacological targets that could specifically mitigate acute disease complications. Retrospective analysis of stored plasma samples from our SCD patients with ACS revealed acute hemolysis, and significantly increased levels of anaphylatoxins (C3a and C5a) and markers of the alternative complement pathway (Ba, Bb) during episodes of ACS compared to their baseline values. To examine the underlying mechanism of the role of complement in ACS, we developed a pre-clinical model of acute lung injury (ALI) in humanized sickle cell (SS) mice. Injection of cobra venom factor (CVF) to SS mice, a commonly used approach to induce complement activation, resulted in rapid deoxygenation, hypopnea and bradycardia (all hallmarks of ALI in mice), followed by death. In contrast, CVF treated littermate control (AA) mice did not develop detectable hemolysis, pulmonary compromise or increase in mortality. The SS mice had markedly increased levels of plasma anaphylatoxin C5a and increased complement component (C3) deposition in kidneys and lungs by immunofluorescence when compared to their controls and those treated with vehicle. While erythrocytes in these SS mice had elevated levels of C3b/iC3b/C3c deposition when compared to AA mice, no difference was noted in the total plasma C3, suggesting sickle erythrocytes are prone to complement-mediated hemolysis.

We then developed a humanized SS x C3 knock-out mice, and preliminary data suggests that these mice are protected from CVF mediated ALI and death. Our data thus far suggest that complement activation in SS mice results in hemolysis, release of free heme and production of C5a, which is a potent pro-inflammatory mediator, all of which possibly play a role in ALI. These results demonstrate that inhibition of C3a or C5a production may represent pharmacological targets to treat ACS in patients with SCD.

B8. Bortezomib Significantly Enhances Gamma Delta T Cell-Mediated Lysis of Acute Myeloid Leukemia and T-cell Acute Lymphoblastic Leukemia

Authors: Story, Jamie; Zoine, Jaquelyn; Burnham, Rebecca; Hamilton, Jamie; Spencer, H. Trent; Doering, Christopher; and Raikar, Sunil

Presenting Author:	**Jamie Story, BS**; jamie.y.story@emory.edu
Type:	Basic - Poster
Poster Available:	Yes - P10
Poster Session Zoom Room Link:	**Visit Story Zoom Room**

Previous studies have shown bortezomib, a proteasome inhibitor, increases surface expression of NKG2D ligands on cancer cells and enhances their sensitivity to innate immune cell-mediated cytotoxicity. Here, we investigate the combination of bortezomib and γδ T cells as a novel therapy for acute myeloid leukemia (AML), a hematologic malignancy in which CAR-based cellular immunotherapy has been challenging. Our results showed 24 hour treatment with bortezomib significantly increased ULBP2/5/6 expression in Kasumi-1 cells and Nomo-1 cells ($n = 3$, $p < 0.05$). Day 12 *ex vivo* expanded γδ T cells were incubated with target cells at the following effector to target (E:T) cell ratios: 0:1, 1:4, 1:2, 1:1, and 2.5:1. Total target cell death of Kasumi-1 cells was significantly increased with bortezomib treatment compared to vehicle control from 16.8% to 52.1% at a 1:4 E:T ratio, 30.0 % to 64.0% at 1:2, and 48.0% to 77.8% at the 1:1 ($n = 3$, $p < 0.05$). Nomo-1 cell death was significantly increased with bortezomib and γδ T cell combination treatment, compared to vehicle control, from 8.8% to 33.7%, 13.9% to 43.6%, 23.1% to 56.5%, and 45.8% to 71.3% at the 1:4, 1:2, 1:1, and 2.5:1 E:T ratios, respectively ($n = 3$, $p < 0.05$). We further validated this combination approach in two T-ALL cell lines, Jurkat and MOLT4, which also had significantly increased surface expression of ULBP2/5/6 with 5 nM bortezomib treatment ($n = 3$, $p < 0.05$). The total cytotoxicity against bortezomib treated Jurkat cells significantly increased from 39.9% to 64.0% at the 1:4 E:T ratio and from to 58.1% to 76.4% 1:2 ratio compared to vehicle control treated cells ($n = 3$, $p < 0.05$). The total cytotoxicity of MOLT-4 cells was also significantly increased with bortezomib treatment compared to vehicle treated cells from 19.6% to 35.5% at the 1:4 E:T ratio, 36.7% to 48.4% at the 1:2 E:T ratio, and 57.4% to 68.3% at the 1:1 E:T ratio ($n = 3$, $p < 0.05$). These results provides proof-of-concept for developing a platform for effective combination therapy of γδ T cells with stress ligand inducing drugs for high-risk leukemias.

B9. Postexposure Prophylaxis to Mitigate the Neurodevelopmental Consequences of Postnatal Zika Virus Infection in Infant Rhesus Macaques

Authors: Raper, Jessica; Schoof, Nils; Richardson, Rebecca; Medina, Alejandra; Rusnak, Rebecca; Kovacs-Balint, Zsofia; Sanchez, Mar; and Chahroudi, Ann

Presenting Author:	**Jessica Raper, PhD**; jraper@emory.edu
Type:	Basic - Poster
Poster Session Zoom Room Link:	**Visit Raper Zoom Room**

Although Zika virus (ZIKV) typically causes mild or no symptoms in adults, infection during pregnancy can result in a spectrum of disease in infants, including birth defects and neurodevelopmental disorders identified in childhood. While intense research has focused on prenatal ZIKV infection, the consequences of postnatal infection in early life are understudied. Using a highly clinically relevant rhesus macaque (RM) model, we have shown that ZIKV infection during infancy negatively impacted brain development resulting in long-term behavioral, cognitive and motor impairments. Considering that ZIKV has infected individuals in 92 countries and is endemic in many areas, it is important to investigate whether a postexposure prophylaxis could mitigate the negative neurodevelopmental consequences of postnatal ZIKV exposure. Using our established postnatal ZIKV RM model, we investigated whether an antiviral treatment could limit viral dissemination into the CNS and alleviate the impact of ZIKV on the developing brain. Three infant RMs received 14-day Sofosbuvir (SOF, 15mg/kg p.o.) treatment starting at 3 days post-infection (dpi). ZIKV+SOF infant RMs were monitored longitudinally for their immune response to ZIKV and SOF treatment, as well as assessing their behavioral, cognitive, motor, and brain development. ZIKV+SOF RMs cleared the virus at a similar rate to ZIKV-infected infant RMs, such that ZIKV was below detection by 7 dpi. Despite similar viral clearance, ZIKV+SOF RMs exhibited social behavior more similar to age- and rearing-matched uninfected controls. Similar to controls, ZIKV+SOF infant RMs exhibited caregiver attachment and prosocial behaviors. However, emotional assessments and neuroimaging suggest an intermittent phenotype. For example, ZIKV+SOF infant RMs exhibited the species-typical response of freezing during the profile condition of the human intruder task, but their level was lower than controls. At 3 months of age, ZIKV+SOF RMs had normal lateral ventricle volumes, but exhibited smaller amygdalae, hippocampi, and total white mater volume compared to controls. The current data suggests that antiviral treatment may help ameliorate some, but not all, of the neurodevelopmental consequences associated with early postnatal ZIKV infection. Further assessments are needed to determine degree that postexposure treatment can alleviate the cognitive and motor impacts of postnatal ZIKV infection.

Poster Presentations: B5 - B10 (In Order by Presenter Last Name)

B10. FVIII-Specific CD4 T Cell Proliferation Requires Multiple Previous Exposures to FVIII

Authors: Zerra, Patricia; Patel, Seema; Baldwin, W. Hunter; Cox, Courtney; Lollar, Pete; Stowell, Sean; and Meeks, Shannon

Presenting Author:	**Patricia Zerra, MD**; pzerra@emory.edu
Type:	Basic - Poster
Poster Available:	Yes - P11
Poster Session Zoom Room Link:	**Visit Zerra Zoom Room**

Factor VIII (FVIII) replacement in hemophilia A can be complicated by neutralizing anti-FVIII IgG alloantibodies that can actively block FVIII activity and prevent optimal replacement efficacy. Currently, no prophylactic therapy prevents inhibitor development, likely due to poor understanding of key immune regulators governing inhibitor formation.

In contrast to other model antigens, inhibitor formation occurs only following multiple FVIII exposures both in humans and mouse models. This suggests that early exposure events may prime subsequent development of long-lasting antibodies. Despite previous studies suggesting that CD4 T cells play an important role in inhibitor development, their timing and overall role in this key immune response remains incompletely understood. Thus, defining the role of CD4 T cells in inhibitor development is important if novel therapeutics for inhibitor prevention are to ever be realized.

As no tools exist to study FVIII specific CD4 T cells, we engineered the model antigen, OVA, into the B domain site of B-domain-deleted FVIII (HOVA) for tracking antigen specific CD4 T cells. HOVA had equivalent FVIII activity and immunogenicity to recombinant FVIII. Additionally, the OVA CD4 T cell epitope in HOVA was functional, as proliferation of CD4 T cells from OTII mice was observed following culture with HOVA *in vitro*. Surprisingly, no proliferation or activation of OTII CD4 T cells was detected in hemophilia A mice immunized with 1-2 HOVA injections. However, in previously highly immunized mice, 1 HOVA injection induced significant proliferation and activation of OTII CD4 T cells.

Understanding initiating immune events in the CD4 T cell-dependent process of FVIII inhibitor development is paramount to the development of novel therapies to prevent inhibitor formation in hemophilia A. HOVA is a unique immunologic tool for examining the FVIII specific CD4 T cell response following FVIII exposure. Using this tool, we found that FVIII specific CD4 T cell proliferation requires more than 2 prior exposures to HOVA, consistent with the observation that multiple FVIII exposures are required prior to inhibitor development. These findings provide an important clue to early steps in the development of FVIII inhibitors, with further studies needed to elucidate the mechanisms underlying this phenomenon.

Posters

All posters provided by abstract authors are available in the following pages. Available posters are labeled P1 - P11.

Achieving Wellness After Illness for Teens (AWAIT): An App-Based Hope Intervention

Karen E. Effinger, MD, MS;[1,2] Rebecca Williamson Lewis, MPH;[2] Betelihem Getachew, MPH;[3] Kuheli Mitra, MPH;[3] Jessica Potts, MPH;[3] Carla J. Berg, PhD, MBA, LP;[4] Ann Mertens, PhD[1,2]

[1]Division of Pediatric Hematology/Oncology/BMT, Emory University School of Medicine, Atlanta, GA; [2]Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, GA; [3]Rollins School of Public Health, Emory University, Atlanta, GA; [4]Milken Institute School of Public Health, George Washington University, Washington, DC

BACKGROUND

- Childhood cancer survivors (CCS) are at increased risk of morbidity and mortality due to treatment-related chronic conditions, which may be augmented by an unhealthy lifestyle.
- Hope, a positive psychological concept focused on goal-directed thinking, has been associated with engaging in health-promoting behaviors.
- Achieving Wellness After Illness for Teens (AWAIT) is a behavioral intervention focused on increasing hope in order to improve quality of life (QoL) and health-promoting behaviors in adolescent CCS.

PURPOSE

- To test the feasibility and acceptability of the AWAIT intervention in CCS.

METHODS

Patient population

- Age 13-17 years
- >3 months from completion of cancer therapy

Randomization

- 2:1 AWAIT intervention vs. attention control
- Stratified by time from therapy completion
  - Early Survivor: 3-36 months off-therapy
  - Late Survivor: >36 months off-therapy
- Stratified by tumor type
  - Leukemia/Lymphoma
  - Brain Tumor
  - Other Solid Tumor

AWAIT Intervention

- 8 weeks
- Mobile app: 8 modules with videos and practice exercises, tracking of mood and healthy lifestyle behaviors, healthy lifestyle tipsheet
- Weekly Coaching Calls to review material and apply new skills

Assessments

- Baseline, 2 months (end of intervention), 4 months
- Revised Children's Hope Scale, PedsQL v4.0, Youth Risk Behavior Surveillance System questions, Acceptability metrics

Analysis

- Fisher's exact, Wilcoxon Rank Sum tests were used to assess differences between groups.
- Paired t-tests and McNemar's tests were used to evaluate changes within groups.

Implementation of the AWAIT intervention is feasible and acceptable to adolescent survivors of childhood cancer.

Survivors receiving the AWAIT intervention showed improvement in aspects of hope specific to goal-directed thinking, increased physical activity and decreased screen time.

RESULTS

Table 1: Demographic & Treatment Characteristics by Treatment Arm

	AWAIT N=32	Control N=16	P-value
Female sex, n (%)	16 (50.0)	6 (37.5)	0.54
Age at enrollment, yrs (med, range)	15 (13-17)	15.5 (13-17)	0.78
Diagnosis, n (%)			0.92
Leukemia/Lymphoma	13 (40.6)	8 (50.0)	
Brain Tumor	6 (18.8)	3 (18.8)	
Other Solid Tumor	13 (40.6)	5 (31.3)	
Age at diagnosis, yrs (med, range)	10 (0-16)	11.5 (1-15)	0.37
Time from therapy completion			1.0
Early Survivor	16 (50.0)	8 (50.0)	
Late Survivor	16 (50.0)	8 (50.0)	

Table 2: Feasibility of the Intervention

	AWAIT N=32	Control N=16
Completion of intervention activities in at least 7 out of 8 weeks	21/32 (66%)*	N/A
Completion 2-month evaluation	21/32 (66%)	13/16 (81%)
Completion 4-month evaluation*	15/24 (63%)	12/14 (86%)

* Pre-COVID19: 20/28 (71%); Post-COVID 19 0/4 (0%)

¥4-month evaluations are still ongoing. Results based on patients who have received 4-month evaluation

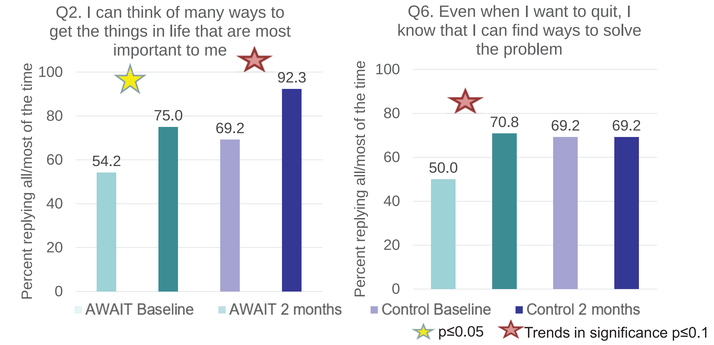
Acceptability

- 72% very/quite satisfied with AWAIT
- 92% would recommend AWAIT to another survivor
- AWAIT participants who felt components were helpful:
  88% coaching, 75% videos, 63% tracking mood, 58% tracking behaviors, 54% practice exercises

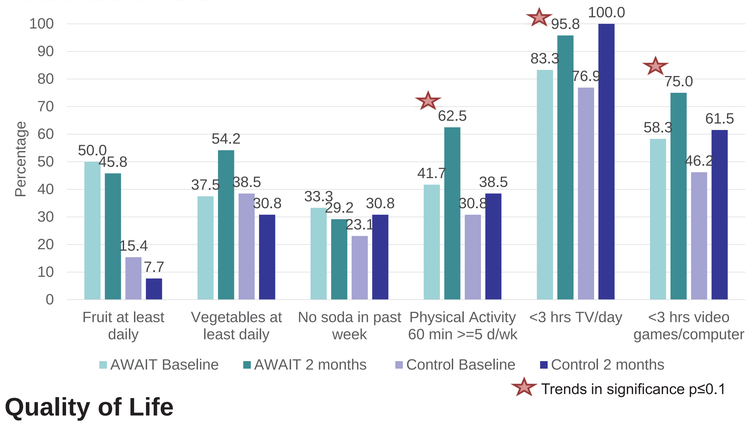
RESULTS

Hope

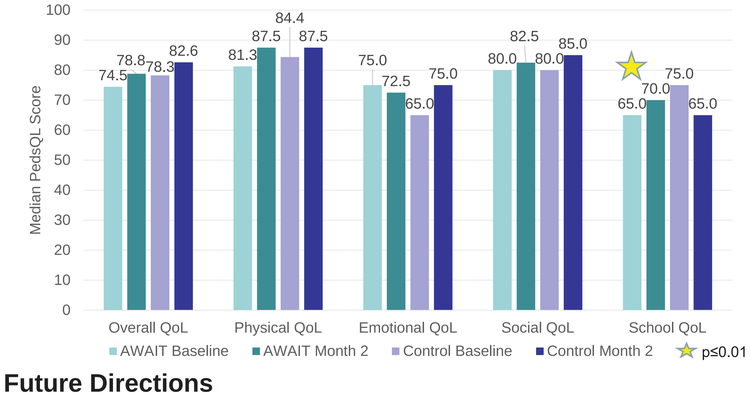
- No change in overall Hope Score (Scale 0-5) for either group (AWAIT 3.8 to 3.9, p=0.13, Control 3.6 to 3.8, p=0.28); however, changes specific in goal-directed thinking were seen.



Health Behaviors



Quality of Life



Future Directions

- We will evaluate for persistence of changes at 2 months after the end of intervention.
- We will compare intervention effectiveness in early and late survivors to determine the ideal time for AWAIT participation.

This work was funded by the Woodruff Health Sciences Synergy Award and the Aflac Pilot Grant.

Quantifying Differences in Risk of Adverse Events by Induction Treatment Regimen in Pediatric Acute Lymphoblastic Leukemia

Zachary West, MD,[1] Sharon M. Castellino, MD, MSc,[1,2] Caitlin Monroe, Ms,[2] Amanda Thomas, MS,[1] Courtney McCracken, PhD,[1] Tamara P. Miller, MD, MSCE[1,2]
[1]Emory University School of Medicine, Atlanta, GA; [2]Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta/Emory University, Atlanta, GA

PURPOSE

- To describe the overall incidence of clinically significant adverse events (AEs), hospital readmission, and ICU admission during the first treatment course (Induction) for *de novo* pediatric Acute Lymphoblastic Leukemia (ALL).
- To compare morbidity by treatment regimen for children during Induction.
- To identify if rates of hospital readmission or ICU admission differed by treatment regimen during Induction.

BACKGROUND

- Advances in therapy for pediatric ALL have led to 5-year survival rates of up to 94%.
- Despites advances, children and adolescents experience AEs during all courses of therapy.
- AEs are identified and graded in pediatric oncology using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) system.
- Treatment regimen during induction is based on NCI risk at diagnosis:
  - NCI High Risk (HR): ≥10 years of age and/or WBC ≥50,000/uL
  - NCI Standard Risk (SR): 3-drug regimen - steroid, vincristine and asparaginase
  - NCI-HR: 4-drug regimen - steroid, vincristine, asparaginase and anthracycline
- There are no predictors of the risk of AEs during Induction.
- There are currently no evidence-based guidelines regarding length of initial hospitalization after diagnosis.

METHODS

- Retrospective cohort study of patients ages 1-21 years with B- or T-cell ALL diagnosed between 1/1/10 and 5/15/18 at Children's Healthcare of Atlanta.
- Developed *a priori* algorithms to identify and grade 20 clinically-significant AEs based on CTCAE v5 definitions.
- Performed manual chart abstraction to capture presence and highest grade of each AE during Induction, hospitalization dates and ICU admissions.
- Chart abstraction of chemotherapy administered was used to characterize Induction treatment regimen as 3-drug or 4-drug.
- Extracted demographic data from the electronic medical record.
- Risk of AEs was compared between Induction treatment regimens in unadjusted and adjusted models using Cox proportional hazard ratios (HR) with 95% CI.
  - Multivariable analyses adjusted for race, ethnicity, body mass index, sex, and central nervous system disease status at diagnosis

RESULTS

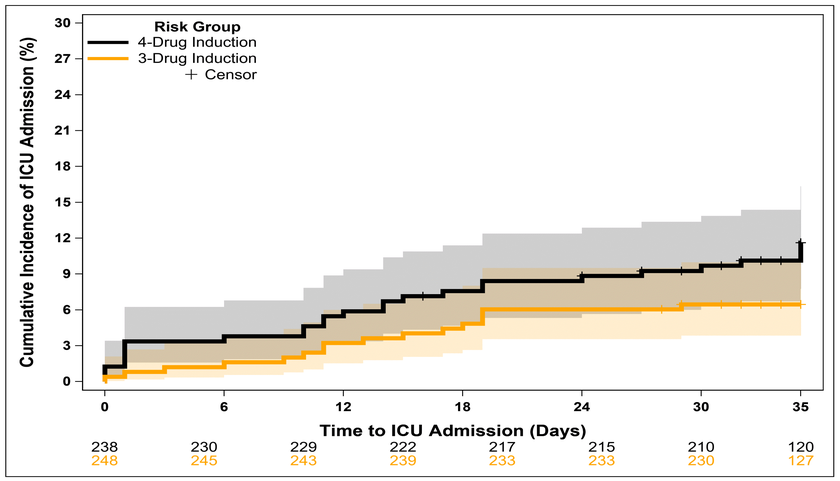
- Of 486 eligible patients, 248 received 3-drug and 238 received 4-drug induction.
  - 45% Female, 58.8% White, 22.8% Hispanic
- Overall, 77.8% (n=378) patients experienced at least one AE.
- Unadjusted risk of multiple AEs differed significantly by treatment regimen. (Table)
- Multivariable analyses:
  - 4-drug: Higher risk of sepsis, hypoxia, hyperbilirubinemia, hyperglycemia, and thromboembolic event
  - 3-drug: Higher risk of hypertension and hepatotoxicity
- Total number of inpatient days differed significantly by treatment regimen (4-drug: 12 days, 3-drug: 4 days, p<0.0001).
- There was no significant difference in rates of hospital readmission (p=0.062) or ICU admission (p=0.117, Figure) by treatment regimen.

Rates and Risks of Clinically Significant AEs by Treatment Regimen

AE	3-drug Induction n (%)	4-drug Induction n (%)	Hazard Ratio[$] (95% CI)	p-value
Fever	61 (24.6)	58 (24.8)	1.00 (0.71, 1.42)	0.991
Infection	81 (32.7)	81 (34.4)	1.05 (0.77, 1.42)	0.775
Sepsis	18 (7.26)	28 (11.8)	1.66 (0.92, 3.00)	0.091
Hypotension	18 (7.26)	26 (10.9)	1.49 (0.82, 2.72)	0.19
Hypertension	73 (29.4)	73 (30.7)	1.04 (0.77, 1.42)	0.786
Hypoxia	14 (5.65)	29 (12.2)	2.24 (1.19, 4.22)	**0.012**
ARDS	0 (0.00)	3 (1.26)	N/A	0.078
Pancreatitis	5 (2.02)	8 (3.36)	1.67 (0.55, 5.10)	0.369
ALT elevation	79 (31.8)	54 (22.7)	0.70 (0.50, 0.99)	**0.041**
AST elevation	21 (8.47)	23 (9.66)	1.15 (0.64, 2.06)	0.646
Hyperbilirubinemia	11 (4.44)	28 (11.8)	2.74 (1.36, 5.49)	**0.005**
Hyperglycemia	12 (4.84)	43 (18.1)	3.91 (2.09, 7.31)	**<0.001**
Thromboembolic Event	3 (1.21)	21 (8.82)	7.58 (2.27, 25.4)	**0.001**
Stroke	2 (0.81)	2 (0.84)	1.05 (0.15, 7.40)	0.908
Neuropathy	22 (8.87)	11 (4.62)	0.51 (0.25, 1.05)	0.066
Seizure	4 (1.61)	6 (2.52)	1.57 (0.45, 5.55)	0.481
Anaphylaxis	0 (0.00)	2 (0.84)	N/A	0.148
Hyponatremia	1 (0.40)	8 (3.36)	8.47 (10.6, 67.4)	**0.044**
Ileus	1 (0.40)	5 (2.10)	5.25 (0.62, 44.7)	0.129
Constipation	0 (0.00)	3 (1.26)	N/A	0.076
Any AE	187 (75.4)	191 (80.3)		0.199

[$] Reference group: 3-drug regimen
AEs ≥ Grade 2: fever, infection, hyperglycemia, thromboembolism; all other AEs were ≥ Grade 3

Time to First ICU Admission by Treatment Regimen



CONCLUSIONS

- Patients receiving 4-drug Induction therapy are more likely to experience clinically significant AEs.
- Patients receiving 4-drug Induction are likely to require a higher total number of hospital than those receiving 3-drug regimens.
- While the lack of difference in hospital readmission rates or ICU admissions may not support longer initial hospitalizations for patients receiving 4-drug Induction, the increased risk of severe AEs indicates strategies to reduce morbidity are needed for this group.

Funding : NCI K07CA211956 (TPM)

Use of Opioids and Benzodiazepines pre/post Analgosedation guideline in the NICU

A Quality Improvement project / Claudia Ocampo, MD; Kaitlin David, PharmD; Martha Wetzel, MSPH; Shannon Hamrick, MD

Introduction

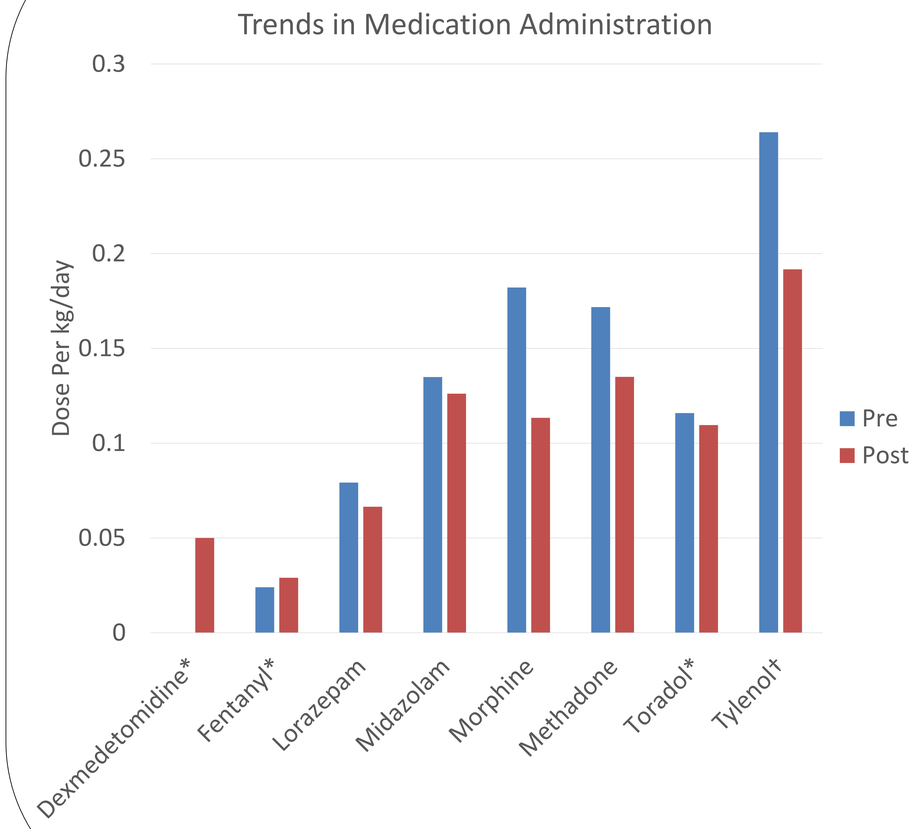
- Surgical NICU patients are frequently exposed to sedatives and analgesics, such as benzodiazepines and opioids.
- An analgosedation guideline for post-surgical patients was created with the goal of decreasing opioid and benzodiazepine use for pain management in the NICU and use of dexmedetomidine as first line agent.
- Dexmedetomidine has been shown to be neuroprotective in animal models.

Methods

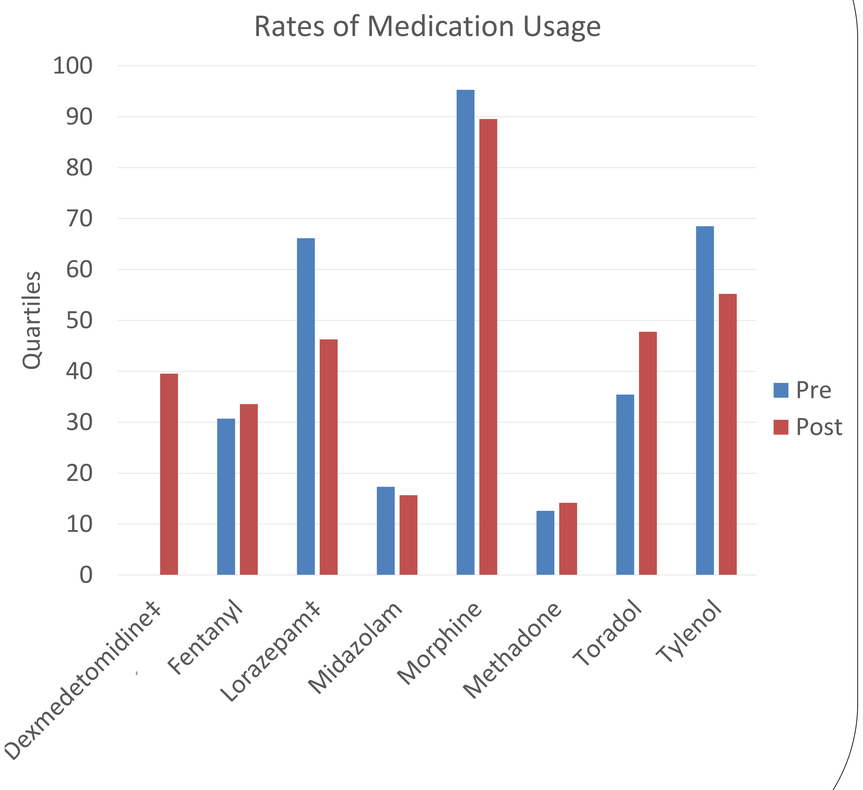
- Retrospective chart review of patients hospitalized prior to and following implementation of analgosedation guideline.
- Patients were grouped as needing light, moderate or both sedation levels according to surgical procedure or intervention.
- Exclusion criteria: did not meet hospitalization dates criteria; incomplete data; unqualifying procedure.
- Utilization of benzodiazepines, opioids, acetaminophen and ibuprofen was compared before and after implementation period.

Drugs that act at the GABA and NMDA receptors have been associated with negative effects on neuroapoptosis and synapse formation during early development in animal models.

Results



*Dose adjusted by factor of 10
†Dose adjusted by factor of 100



‡Statistically Significant

Conclusions

No statistically significant differences were observed in the doses of the medications. A higher percentage of patients received **dexmedetomidine** and toradol in the post period compared with the pre period, and a lower percentage received **lorazepam** and Tylenol. Adjustment for procedure count did not change the statistical significance of any of the outcomes.

Investigating the Contribution of Unique Risk and Protective Factors Associated with Health-related Quality of Life in Newly Diagnosed Pediatric Inflammatory Bowel Disease

Bonney Reed[1], PhD; Sharon Shih[2], MS, Grace Cushman[3], MS, and Subra Kugathasan[1], MD

[1] Emory+Children's Pediatric Institute, [2] Georgia State University, [3] University of Georgia

This research was supported by the National Center for Advancing Translational Science (NCATS) of the National Institutes of Health under Award Number UL1TR002378 and KL2TR002381 and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health under Award Number K23DK122115 awarded to the first author.

Background and Objectives

- Health-related quality of life (HRQOL) in pediatric inflammatory bowel disease (IBD) is typically examined from a deficit standpoint
- Little is known about emotional factors associated with resilient HRQOL outcomes
- Emotional reactivity and adaptability are temperamental characteristics
  - Emotional reactivity is a dispositional attribute characterized by emotional responses that are intense, peak rapidly, and/or are slow to return to baseline
  - Adaptability refers to the ability to quickly and easily adjust to changes in environment or unexpected circumstances
- Aims were to (1) characterize emotional reactivity and adaptability, and (2) use a risk and resilience framework to investigate how emotional reactivity and adaptability are related to parent and child ratings of child HRQOL in a sample of youth with newly diagnosed IBD

Experimental Methods

- Participants included 54 caregivers and 56 youth ages 8 to 17 diagnosed with IBD, recruited in clinic within 45 days of diagnosis
- Measures
  - Child HRQOL using the IMPACT-III (Otley et al., 2002), a disease-specific measure
  - Emotional Reactivity, Parent Report of Children's Reactions (Reynolds & Alfano, 2016)
  - Adaptability Subscale, BASC-2 Parent Report (Reynolds & Kamphaus, 2004)
  - Clinical disease activity, physician global assessment
    - Male participants endorsed significantly higher total HRQOL (male M = 142.84, SD = 11.84, female M = 125.71, SD = 19.65, t = -3.83, p < .001, d = 1.06)
    - Parents reported greater emotional reactivity for their female children compared to males (female M = 30.97, SD = 6.54, male M = 26.87, SD = 6.59, t = 2.25, p = .03, d = 0.62)
    - Parents on average rated their children with IBD as demonstrating typical levels of adaptability based on age and sex norms available (M = 50.27, SD = 9.99)
- Multiple linear regression analyses were conducted to examine the associations between total HRQOL and risk (emotional reactivity) and protective (adaptability) variables.

Results

Multiple regression analyses examining the effects of Demographic, Medical, and Psychosocial Variables on Child-reported and Parent-reported HRQOL

Dependent Variable and Predictors	B	SE B	β	t	F	R^2	ΔR^2
Child-reported HRQOL							
Demographics					17.14^{***}	.26	
Child Sex	13.15	2.99	$.35^{***}$	4.39^{***}			
Medical Variables					42.71^{***}	.64	.38
Disease Symptoms	-1.25	.16	$-.63^{***}$	-8.09^{***}			
Psychological Risk					34.30^{***}	.68	.05
Emotional reactivity	-.37	.23	-.14	-1.58			
Protective Factor					31.33^{***}	.73	.05
Adaptability	.43	.16	2.80^{**}	2.80^{**}			
Parent-reported HRQOL							
Demographics					7.11^{**}	.13	
Child Sex	8.17	4.25	.22	$1.92^{\dagger}$			
Medical Variables					6.44^{**}	.21	.08
Disease Symptoms	-.59	.22	-.30	-2.70^{**}			
Psychological Risk					6.82^{**}	.30	.09
Emotional reactivity	-.43	.33	-.16	-1.29			
Protective Factor					9.01^{***}	.43	.14
Adaptability	.74	.22	.40	3.35^{**}			

Note. Italicized variables are dependent variables. $^{\dagger}p < .10$, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

Racial and Socioeconomic Disparities in Non-Accidental Trauma Screening

Hayley Sooknarine & Edward Richer

Background: It has been found that minority children undergo screening more frequently for non-accidental trauma (NAT). This can be the case despite controlling for expert opinion on NAT likelihood.

Aims: 1) Evaluate NAT screening practices of young infants presenting with apparently isolated skull fractures, and 2) identify which infants have a high level of concern for NAT.

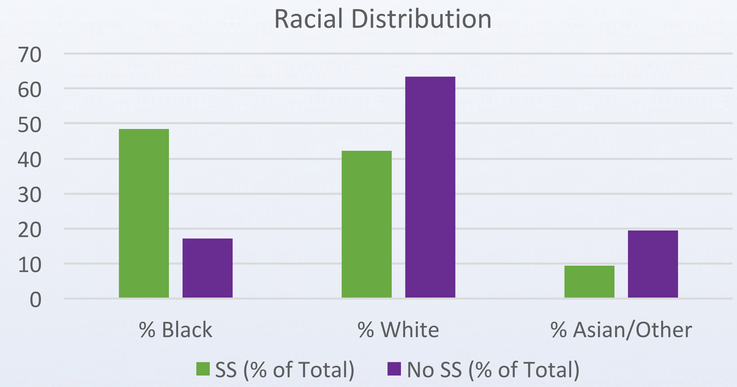
Methods: This was a retrospective chart review. Inclusion criteria were patients under 6 months of age presenting to 2 metropolitan Pediatric trauma centers (one academic and one non-academic) with simple skull fractures between January 2014 and December 2016. Patients were excluded if there was high level of concern for NAT due to history, examination findings, or additional head CT findings.

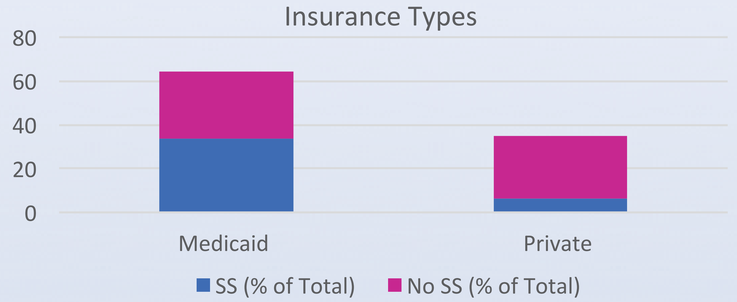
The study population was divided into 2 groups: infants undergoing skeletal surveys (SS) versus not. Demographic information was collected, and information was obtained on history of presentation and SS results. Data analysis was performed using χ^2, Fisher exact test, and odds ratio (OR) calculations.

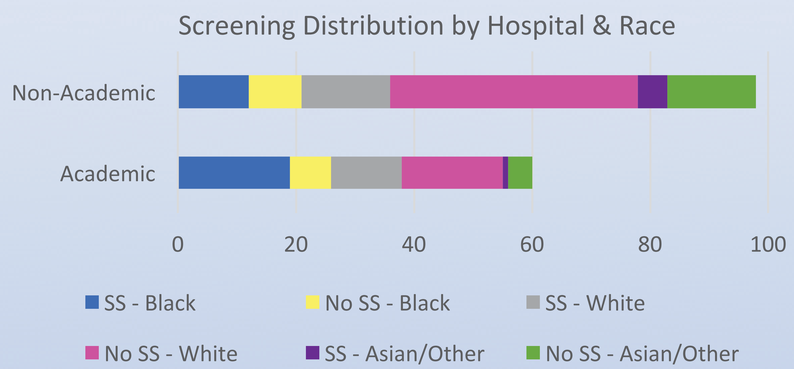
Results: 157 infants were included. 54.8% were white, 29.9% were black, and 15.3% were Asian/other. 64.3% were publicly insured and 35.7% were privately insured. It was noted that of the excluded patients, at least 2 had isolated simple skull fractures due to inflicted injury (ex. hit during parental intimate partner violence). 40.8% underwent NAT screening with skeletal survey, and 1 of these surveys showed additional fractures which increased the level of concern for NAT (1.6% of those screened). The infant with the positive SS had no history to explain the fracture.

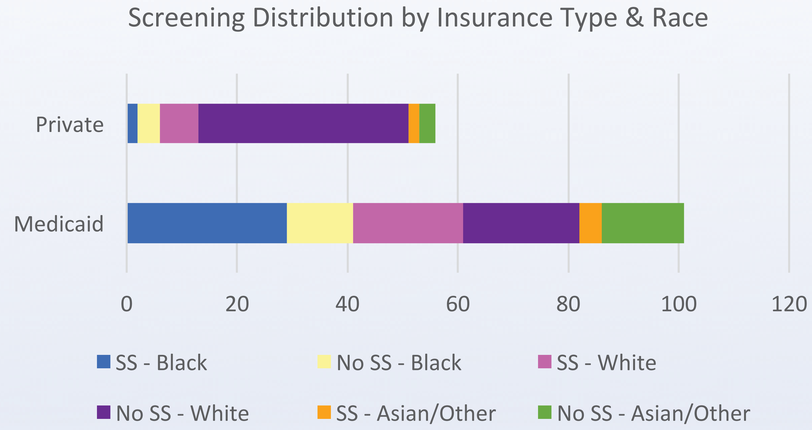
66% of black infants were screened, 31.4% of white infants were screened, and 25% of Asian/other infants were screened (P<0.001). 32.1% of Hispanic infants were screened. The academic hospital screened 53% of presenting infants, while the non-academic hospital screened 33% (P = <0.001). Both hospitals screened higher percentages of black infants compared to other races. 73.1% of black infants were screened at the academic hospital, versus 57.1% at the non-academic hospital (P = 0.05). Conversely, 26.3% of white infants were screened at the non-academic hospital compared to 41.4% at the academic hospital (P = <0.001).

87.2% of black infants were publicly insured versus 47.7% of white infants and 79.2% of Asian/other infants. 89.3% of Hispanic infants were publicly insured. Of the publicly insured, 70.7% of black infants were screened versus 48.8% of white infants and 21% of Asian/other infants (P=0.001). 36% of publicly insured Hispanics were screened.









The odds ratio for being screened if publicly insured was 4.517 (CI: 2.0993 to 9.7191, P=0.0001). Of the privately insured patients, less than 50% of patients in all races underwent skeletal surveys (2 of 6 (33.3%) black, *7 of 45 (15.6%) white*, 2 of 5 (40%) Asian, P=0.2).

Both hospitals saw lower numbers of privately insured patients; however, a smaller proportion of privately insured patients were surveyed at the non-academic hospital (6 of 38 (15.8%)) compared to the academic hospital (5 of 18 (27.8%)) (P<0.0001).

Discussion: This retrospective chart review demonstrated multiple disparities: 1) Racial disparity; 2) Socioeconomic disparity; and 3) variation in screening practices between 2 metropolitan Pediatric trauma centers (academic versus non-academic). Of the publicly insured, more black infants were screened compared to white and Asian/other infants. When comparing the 2 facilities, less privately insured patients were surveyed at the historically private facility. It was also noted that of the private patients, a smaller proportion of white infants were screened compared to black and Asian infants, although the latter groups were much smaller.

Conclusions: There were racial, socioeconomic, and center-to-center disparities in screening for simple skull fractures. These fractures have a low specificity for physical abuse but can occur due to inflicted trauma (as noted for some excluded patients in this study). When controlling for each variable (race and insurance type i.e. socioeconomic status), disparities persisted.

These findings support a need for standardized protocols, especially for injuries of lower specificity for NAT.

Impact of gut microbiome changes on hematopoietic stem cell transplantation outcomes in children.

Mehgan Teherani[1,2]; Zoe Pratte[3]; Samridhi Banskota[1]; Dalia Gulick[3]; Naima Djeddar[4]; Scott Gillespie[1]; John Horan[1,2]; Gregory Gibson[3]; Muna Qayed[1,2]

[1]Emory University School of Medicine, Atlanta, GA [2]Children's Healthcare of Atlanta, Atlanta, GA; [3]Department of Biological Sciences, Georgia Institute of Technology,, Atlanta, GA [4]Molecular Evolution Core, Georgia Institute of Technology, The Center for Integrative Genomics, Atlanta, GA

Background

- Higher gut microbiome diversity in adult allogeneic hematopoietic cell transplantation (HCT) recipients is associated with reduced bloodstream infections and improved overall survival.
- In single center studies, antibiotic exposure through engraftment has been linked to reduced short chain fatty acid (SCFA) producing organisms.
- We examine changes in microbiome in a cohort of pediatric patients undergoing HCT.

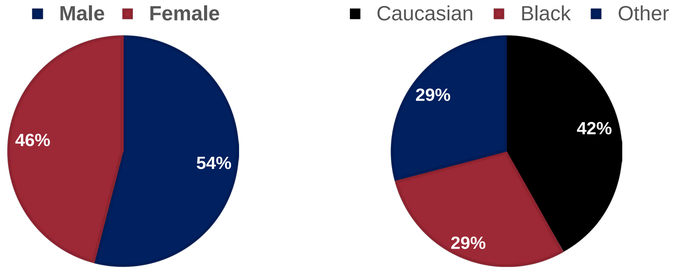
Methods

- Children (<21 years) undergoing allo-HSCT for a hematologic malignancy, using myeloablative conditioning, between 2013-2020.
- Patients were enrolled in an institutional biorepository (n=82) with a subset enrolled in an ongoing trial using rifaximin prophylaxis (n=21).
- Primary outcome: Death at 1 year post-HCT.
- Timing of systemic antibiotics for all patients: none, early (≤D0, day of graft infusion), and late (>D0).
- 16S rRNA sequencing of stool samples obtained at baseline, D0 to D+28, weekly.

Results

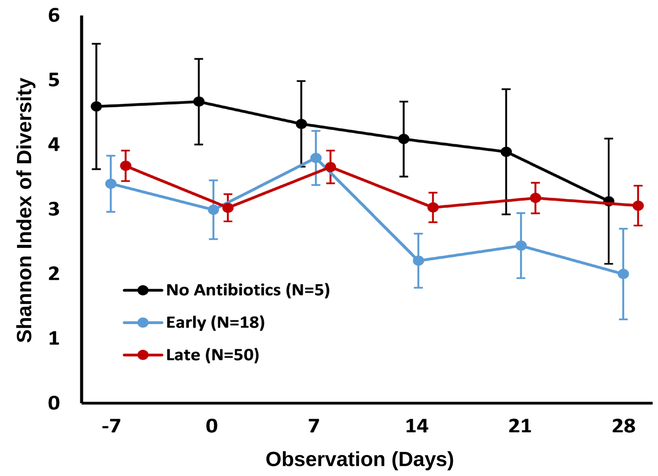
Clinical characteristics

• Median age was 9 years old (range 1-20).



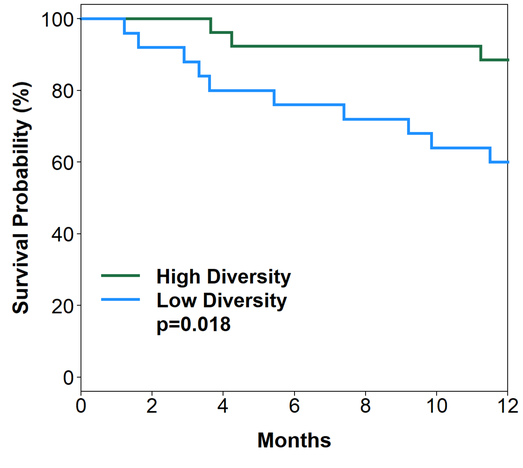
Microbiome diversity changes significantly over time

- Diversity decreased significantly for all patients in the first month post-HCT (p=0.008).
- Piperacillin-tazobactam was used empirically in 91% of patients.
- Higher diversity was seen when patients received none or late versus early antibiotics, but this was not statistically significant, p=0.23.



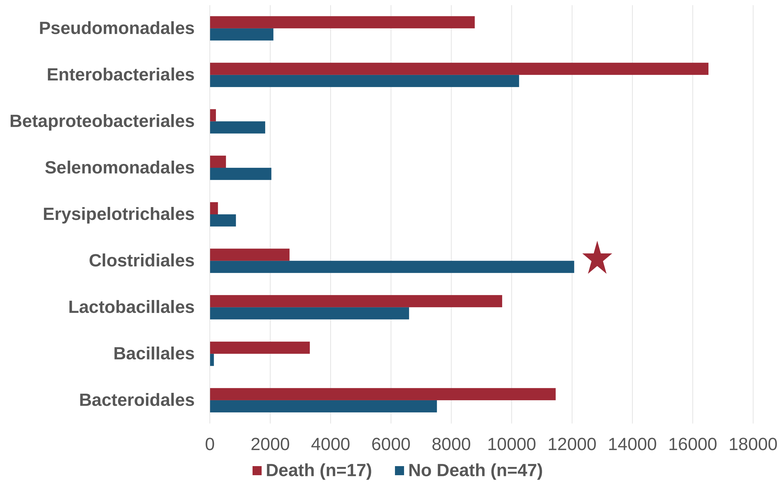
Microbiome diversity at D28 post HCT correlates with overall survival

- OS at 1 year was 88.5% for patients with high diversity (≥median) versus 60% with low diversity.



Significant depletion of Clostridiales among patients who died when compared to survivors

- There was a significant difference in absolute abundance of Clostridiales order among survivors and non-survivors (p=0.0045).
- Other observed expansion of pathogenic orders such as Pseudomonadales, Enterobacteriales, Bacillales, Bacteroidales was not statistically significant.



★ Denotes significant difference (p=0.0045) of Clostridiales order

Conclusions

- Early antibiotic exposure is detrimental to microbiome diversity.
- Microbiome diversity at engraftment is strongly correlated with survival.
- Higher quantity of SCFA producing bacteria, Clostridiales, is associated with improved survival.
- Approaches to preserve microbiome diversity, such as limiting anaerobic antibiotic exposure, may improve SCT outcomes.
- Our ongoing study will provide preliminary data on the impact of rifaximin on preserving microbiome diversity.

References:
1) Taur, Y., et. al. *CID*. 55(2012): 905-914.
2) Weber, D., et. al. *Biol Blood Marrow Transplant*. 23(2017): 845-852.
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Use of a Novel Human Milk-Based Fortifier in Term Infants with Surgical Congenital Gastrointestinal Disorders Improves Growth at Discharge

Sarah M. Tweddell, MD, Maria E. Barbian, MD, Heidi E. Karpen, MD, Emory University, Children's Healthcare of Atlanta

Introduction

- Human milk (HM) is the ideal source of nutrition for all infants, but alone is insufficient in protein and calories for infants with surgical congenital gastrointestinal disorders (S-CGD) and critically ill infants.
- Currently, the only option for term infants is fortification with formulas which have been associated with increased risk of necrotizing enterocolitis (NEC) in the preterm population and may contribute to the high rates of NEC in those infants with congenital gastrointestinal disorders.
- A human milk-based fortifier (HMBF) formulated for term infants with S-CGD is needed to provide optimal nutrition.
- The purpose of this study is to demonstrate the safety and efficacy of a novel HMBF designed for term infants with S-CGD.

Methods

- Patients were fed an exclusive human milk diet consisting of mother's own milk (MOM), and pasteurized donor human milk (PDHM) fortified with a novel HMBF (PBCLN-002, Prolacta Bioscience) specifically formulated for the term infant.
- The first 26 term infants enrolled in this trial were evaluated for safety and efficacy of PBCLN-002 compared to a recent cohort of patients fed diets containing MOM and formula.
- The retrospective cohort was matched based on diagnosis, sex, birthweight and gestational age.

In patients with surgical congenital gastrointestinal disorders, a human-milk based fortifier demonstrates safety and efficacy by increased growth at the time of discharge.

Results

- There were no adverse events in the PBCLN group and no significant effect on feeding tolerance, interruptions or maximum direct bilirubin.
- Infants receiving PBCLN-002 grew faster in terms of weight and length and head circumference velocity; however, these were not statistically significant.
- Adjusting for baseline using a basic analysis of covariance model, these differences remained significant for discharge weight (p=0.02) and discharge length (p=0.003), and marginally for discharge head circumference (p=0.13).

Table 1. Infant Demographics and Baseline Characteristics.

	PBCLN (n = 26)	Control (n = 26)
Birth Weight (g)	2874 ± 468	2833 ± 564
Birth Length (cm)	47.6 ± 3.0	47.2 ± 3.1
Birth FOC (cm)	33.3 ± 1.6	32.9 ± 1.4
Female (%)	15 (57.7%)	14 (53.8%)

Birth measurements are reported in mean ± SD.

Table 2. Outcomes.

Measurements are reported as mean ± SD (median). The 95% normal-theory CI is also reported. Wilcoxon signed-rank test was run to generate the p-value.

		PBCLN (n = 26)	Control (n = 26)	p-value	95% CI (Prolacta-Control*)
Feeding Intolerance (Days)	≥ 1 0 Median ± IQR	5 21 0 ± 0	6 20 0 ± 0.25	0.69	
Feeding Interruptions (Days)	≥ 1 0 Median ± IQR	8 18 0 ± 1	5 21 0 ± 0	0.35	
Maximum direct bilirubin (mg/dL)		1.08 ± 1.12 (0.65)	0.95 ± 1.21 (0.60)	0.31	-0.45 to 0.72
Weight gain (g/day)		24.4 ± 12.6 (25.5)	19.5 ± 10.5 (22.1)	0.30	-3.0 to 12.9
Weight gain (g/kg/day: exponential)		7.2 ± 3.3 (8.0)	6.5 ± 3.8 (6.5)	0.76	-1.5 to 3.1
Discharge weight (g)		3935 ± 776 (3892)	3463 ± 1144 (3156)	0.03	-61.4 to 1005.2
Length velocity (cm/week)		0.86 ± 0.40 (0.90)	0.73 ± 0.66 (0.80)	0.44	-0.15 to 0.42
Discharge length (cm)		52.7 ± 2.4 (53)	50.3 ± 4.2 (49)	0.025	0.44 to 4.32
Head circumference velocity (cm/week)		0.50 ± 0.27 (0.55)	0.55 ± 0.28 (0.49)	0.78	-0.20 to 0.10
Discharge head circumference (cm)		36.4 ± 1.9 (36.4)	35.2 ± 2.4 (34.8)	0.052	-0.003 to 2.37

Conclusions

- In this cohort of patients with S-CGD, PBCLN-002 demonstrates safety and efficacy by increased growth at the time of discharge.
- The non-significant differences in daily weight gain and growth velocities between these two groups may reflect the small sample size and differences in parenteral nutrition support during the early phases of surgical recovery.

Parent Psychological Distress and Chronic Pain in Pediatric Sickle Cell Disease

Kerri E. Woodward, PhD.[1], Soumitri Sil, PhD.[1], Yelena L. Johnson, PhD.[1], Carlton Dampier, MD[1], Lindsey L. Cohen, PhD.[2]
[1]Aflac Cancer and Blood Disorders Center of Children's Healthcare of Atlanta, Division of Pediatrics, Emory University School of Medicine
[2]Georgia State University, Department of Psychology

Background

- Chronic pain occurs in approximately 20-40% of adolescents and adults with sickle cell disease (SCD), which is correlated with decreased quality of life and physical functioning and more anxiety/depression symptoms[1-3].
- Pediatric chronic pain is associated with increased parenting distress and poor emotional and family functioning[4,5].
- Although parents' distress is an appropriate emotional response to a child's chronic medical condition, it has also been associated with poorer child outcomes, including increased disability and reduced quality of life[5,6].
- Despite studies supporting the influence of parental distress and child outcomes in medical populations, research is needed to investigate these psychosocial influences in SCD.
- This study aimed to investigate how parenting stress, parent emotional functioning, and family functioning 1) were associated with child outcomes and 2) differed based on the chronicity of pediatric SCD pain.

Hypotheses

For children and adolescents with SCD, parenting stress, parent emotional functioning (e.g., anxiety, depression), and family functioning will:

- **Be associated with child outcome.** More parenting stress, anxiety, and depression and poorer family functioning will be associated with poorer child quality of life and greater child functional disability.
- **Differ based on chronicity of child SCD pain** Parents of youth with chronic pain will experience more stress, anxiety, and depression and poorer family functioning than parents of youth with episodic pain.

Method

Participants

- 77 youth with SCD
- Ages 9-20 years; M_{age}=14.2 years (*SD*=2.5)
- 57% Female
- 93% African American, 3% Biracial, 1% Caucasian
- 75% HbSS, 13% HbSC, 9% HbSB^{+}thal, 1% HbSB^{0}thal
- 81% prescribed Hydroxyurea
- 15% receiving chronic transfusions
- 44% chronic pain, 55% episodic pain

Method (Continued)

Procedure

- Participants completed validated measures during an SCD outpatient clinic visit or at home via paper-pencil or web-based (REDCap®).

Measures

- Parent Measures
  - Pediatric Inventory for Parents[7,8]
  - Center for Epidemiological Study – Depression Scale Revised[9-13]
  - Generalized Anxiety Disorder Scale[14-17]
  - Family Assessment Device[18-22]
- Child Measures
  - Pain Characteristics: Average pain intensity (0-10), pain frequency (0-30 days/month), and pain duration[1,2]
  - Functional Disability Inventory[23-25]
  - Pediatric Quality of Life – SCD Module[26-29]

Results

Correlations Among Parent Functioning and Child Functioning

	Child Functioning	
Parent Variables	Functional Disability	Quality of Life
Parenting Stress Frequency	0.42***	-0.40***
Parenting Stress Difficulty	0.34**	-0.40***
Depressive Symptoms	0.34**	-0.26*
Anxiety Symptoms	0.26*	-0.25*
Family Functioning	0.01	-0.24*

*p<.05, **p<.01, ***p<.001

- Greater parenting stress frequency, greater parenting stress difficulty, greater parent depression, and greater parent anxiety were associated with increased child functional disability (p's<.05).
- Greater parenting stress frequency, greater parenting stress difficulty, greater parent depression, and greater parent anxiety were associated with poorer child quality of life (p's<.05).
- Poorer family functioning was associated with poorer child quality of life (p<.05).

Results (Continued)

- Parents of children with chronic pain endorsed significantly more frequent and difficult parenting stress than parents of children with episodic pain (p's<.01).
- Parents of children with chronic pain endorsed more symptoms of anxiety than parents of children with episodic pain (p=.03).
- Depressive symptoms and family functioning did not differ between parents of children with episodic versus chronic pain (p's>.05).

Mean (M) and Standard Deviation (SD) Scores on Parent Functioning for Youth with Episodic and Chronic Sickle Cell Pain

	Child Episodic Pain M (SD)	Child Chronic Pain M (SD)	t-value [95% CI]	p-value
Parenting Stress Frequency	83.88 (31.43)	121.26 (30.51)	-5.06 [-52.11, -22.65]	<.01
Parenting Stress Difficulty	73.0 (29.45)	100.61 (40.47)	-3.30 [-44.29, -10.93]	<.01
Depressive Symptoms	7.76 (6.51)	9.13 (6.27)	-0.90 [-4.42, 1.67]	.37
Anxiety Symptoms	3.51 (3.94)	6.03 (5.78)	-2.20 [-4.81, -0.23]	.03
Family functioning	1.67 (0.59)	1.50 (0.39)	-1.51 [-0.41, 0.06]	.14

Conclusions

- Pediatric pain frequency in SCD is associated with increased parenting stress.
- Additionally, increased parenting stress, anxiety, and depression and poorer family functioning are associated with poorer child outcomes (i.e., quality of life, functional disability).
- Further studies are needed to investigate parent-focused interventions to manage stress, emotional functioning, and family functioning for parents of children with chronic pain.

Poster and references are available upon request.

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Bortezomib Significantly Enhances γδ T Cell-Mediated Lysis of Acute Myeloid Leukemia and T-Cell Acute Lymphoblastic Leukemia

Jamie Y. Story, B.A.[1,3], Jaquelyn T. Zoine, B.S.[2,3],, Rebecca E. Ryan, B.S.[1,3], Jamie A. Hamilton[2,3], H. Trent Spencer, Ph.D.[1,3], Christopher B. Doering, Ph.D.[1,3] and Sunil S. Raikar, M.D.[3]

[1]Graduate Program in Molecular and Systems Pharmacology, Emory University, Atlanta, GA, [2]Graduate Program in Cancer Biology, Emory University, Atlanta, GA, [3]Aflac Cancer and Blood Disorders Center, Department of Pediatrics, Emory University, Atlanta, GA

Contact information: jystory@emory.edu

Background

- Relapse still remains a clinical barrier in the field of childhood leukemia
  - Allogeneic hematopoietic stem cell transplantion (HSCT) is only realistic chance at a cure for relapsed AML and T-ALL, but requires patients to be in remission
  - CAR T cell therapy has been difficult to adapt in both leukemias due to on-target, off-tumor effects
  - Other immunotherapies need to be explored for AML and T-ALL
- γδ T cells are an attractive candidate for cancer immunotherapy
  - Can induce cell lysis through engagement with stress antigens on tumor cells, such as natural-killer group 2, member D (NKG2D) ligands
  - Our lab previously developed a serum free GMP method for *ex vivo* expansions of γδ T cells (Vγ9Vδ2)
- Certain drugs can increase stress antigens on cancer cells
  - Bortezomib previously shown to increase NKG2D ligands on AML cells
  - Limited benefit from addition of bortezomib to standard chemotherapy in clinical setting for AML

Hypothesis

We hypothesize a more effective approach is a combination of bortezomib with *ex vivo* expanded γδ T cells, potentially as a bridge to transplant by inducing remission

Research Design

- Incubated Kasumi-1, Nomo-1, Jurkat, and MOLT-4 cells with 2.5, 5, and 10 nM of bortezomib over 48 hours to determine optimal drug treatment for increase in NKG2D ligands' surface expression
- Treated Kasumi-1, Jurkat and MOLT-4 cells with 5 nM bortezomib and Nomo-1 cells with 10 nM bortezomib or vehicle control for 24 hours prior to a 4 hour *in vitro* cytotoxicity assay with *ex vivo* expanded γδ T cells at various effector to target (E:T) ratios
  - Measured target cell death by flow cytometry via 7-AAD and Annexin V staining
- Assessed whether bortezomib treatment had negative effects on γδ T cells
  - Treated γδ T cells with 1-5 nM bortezomib for 24 hours, which is range of expected plasma concentration 24 hours after bortezomib injection in adults
- Incubated GFP$^+$ target cells with γδ T cells at lower E:T ratios over 48 hours to assess if bortezomib treatment accelerated target cell death over a longer time period

Results

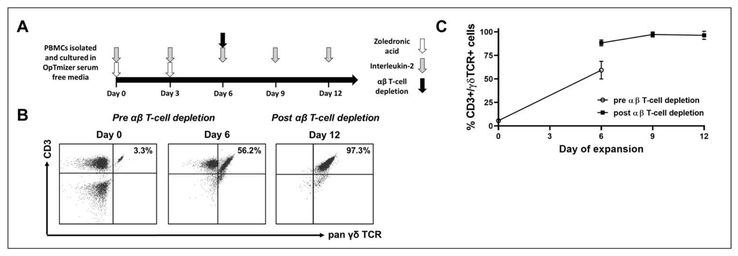

Figure 1. Expansion of γδ T cells from healthy donor PBMCs. (A) Healthy donor PBMCs were cultured in serum free media over a day 12 period. Cultures were supplemented with 500 IU IL-2 and 5 μM zoledronic acid on days 0 and 3, and 1000 IU IL-2 on days 6, 9, and 12. On day 6, cultures were depleted of αβ T cells using positive αβ T cell selection. B. (n = 6 different donors). (B) Representative flow of the percentage of γδ T cells over the course of expansion for one donor. Depleting cultures of αβ T cells yielded a highly pure γδ T cell product by day 12 of expansion. (C) Flow cytometry was performed every 3 days to monitor the percentage of γδ T cells over the expansion. Live cells were gated on and γδ T cell percentage was determined by CD3$^+$/γδ TCR$^+$. Post-αβ depleted cultures were 88.3 ± 3.1% γδ T cells, compared to 59.3 ± 9.4% prior to depletion on day 6 (n = 6 separate donors). By day 12 of expansion, all cultures had an average of 96.4 ± 4.3% γδ T cells (n = 6 separate donors).

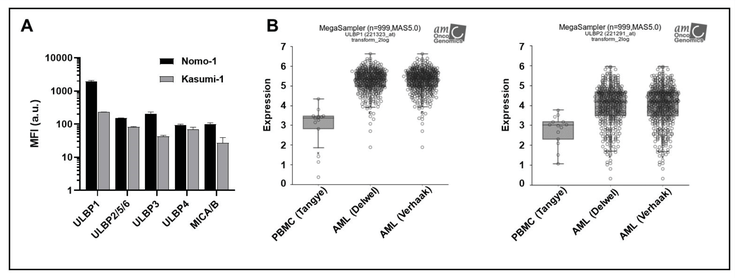

Figure 2. Expression of NKG2D ligands in AML cell lines and primary AML patient samples. (A) Baseline protein expression of NKG2D ligands on AML cell lines was assessed by gating on Annexin V and 7-AAD double negative cells and analyzing surface marker expression via flow cytometry. Both AML cell lines showed highest expression of ULBP1 (n = 3, 1934.0 ± 126.2 MFI arbitrary units for Nomo-1 and 230.0 ± 3.5 MFI arbitrary units for Kasumi-1). (B) RNA sequencing data from primary AML patient samples showed elevated gene expression in ULBP1 and ULBP2 compared to gene expression in normal PBMCs.

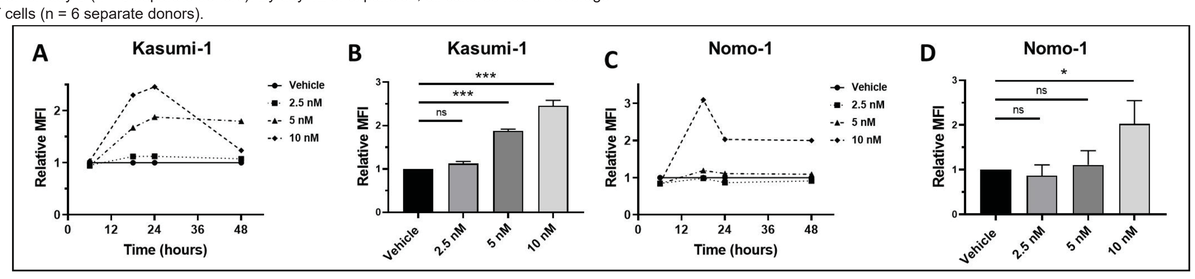

Figure 3. Bortezomib significantly increases expression of ULBP2/5/6 in AML cell lines after 24 hours of treatment. (A and C) AML cell lines were treated with vehicle control or increasing doses of bortezomib for 48 hours. Live cells were gated on (7-AAD$^-$/Annexin V$^-$) and analyzed for changes in NKG2D ligand surface expression at the various time points and concentrations (n = 3, average relative MFI). Peak expression of ULBP 2/5/6 was seen between hour 18 and 24 in both cell lines. (B) Surface expression of ULBP2/5/6 on Kasumi-1 cells significantly increased with 5 nM and 10 nM bortezomib treatment (n = 3, $p < 0.0001$ for both groups). (D) Nomo-1 cells had a significant increase in ULBP2/5/6 at the 10 nM dose (n = 3, $p = 0.0124$). Data are displayed as an average +/- SD of relative MFI of bortezomib treated cells compared to vehicle control treated cells. Significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.

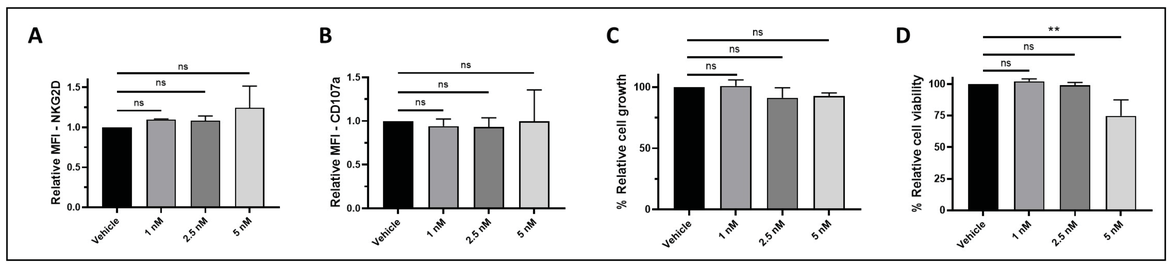

Figure 4. Bortezomib has no major negative effects on γδ T cells. γδ T cells were treated with 1, 2.5, or 5 nM bortezomib for 24 hours at day 10 of the expansion. There was no significant decrease in surface expression of NKG2D receptor (A) and CD107a (B) on live γδ T cells (7-AAD$^-$/Annexin V$^-$) at any bortezomib dose (n = 3 separate donors, $p > 0.05$). (C) Bortezomib treatment had no negative effect on cell growth (n = 3 separate donors, $p > 0.05$). (D) There was a significant decrease in cell viability at 5 nM, from 98.9% to 74.8% ($p = 0.004$, n = 3 separate donors). Data are displayed as an average +/- SD of relative MFI of bortezomib treated cells compared to vehicle control treated cells. Significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.

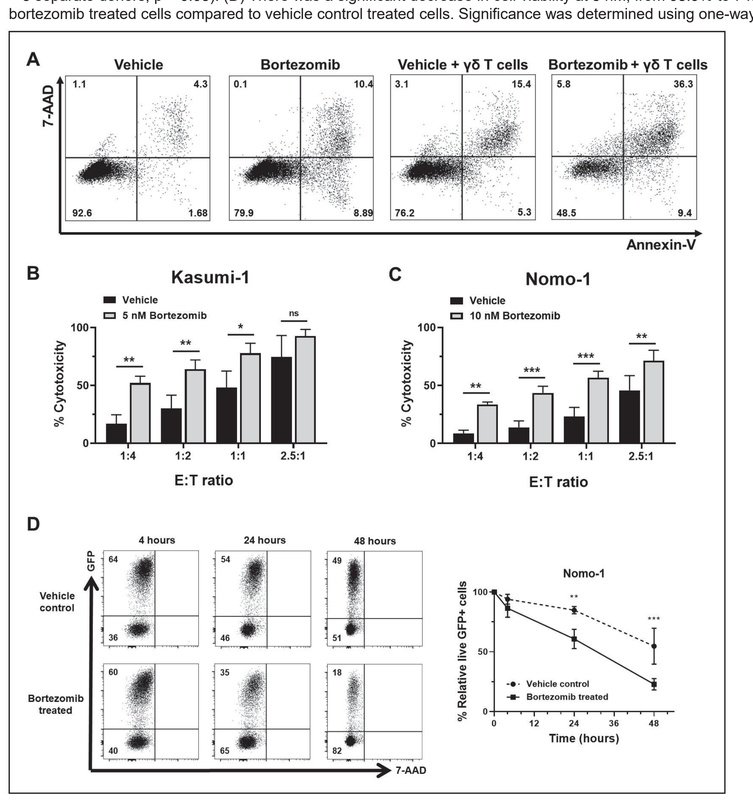

Figure 5. Bortezomib enhances cytotoxicity of γδ T cells against AML cell lines. (A) Representative flow plots from a 4 hour cytotoxicity assay with γδ T cells and Nomo-1 cells at a 1:1 effector to target (E:T) ratio. After 24 hour treatment with bortezomib or vehicle, AML cells were incubated with day 12 ex vivo expanded γδ T cells at 1:4, 1:2, 1:1, and 2.5:1 E:T ratios for 4 hours. The difference in cytotoxicity of γδ T cells against bortezomib-treated target cells and vehicle-treated target cells was analyzed at each E:T ratio. Replicates represent γδ T cells expanded from 3 different donors. (B) Treating Kasumi-1 cells with bortezomib significantly increased target cell death at the 1:4, 1:2, and 1:1 E:T ratios (n = 3, $p = 0.0044$, 0.0058, and 0.016, respectively). (C) Cytotoxicity of Nomo-1 cells significantly increased in bortezomib-treated cells compared to vehicle-treated cells at all E:T ratios (n= 3, $p = 0.0025$, 0.0005, 0.0001, and 0.0021, respectively). (D) Representative flow plots from a longer time course cytotoxicity assay with γδ T cells and Nomo-1 cells at a 1:2 E:T ratio. After 24 hours of treatment with bortezomib or vehicle, GFP$^+$ Nomo-1 cells were incubated with day 11 ex vivo expanded γδ T cells for 4, 24, and 48 hours. The percentage of remaining live GFP$^+$ cells (GFP$^+$/7-AAD$^-$) was determined at each time point to determine whether bortezomib treatment increased the cytotoxicity of GFP$^+$ Nomo-1 cells compared to vehicle-treated cells. Bortezomib-treated Nomo-1 cells incubated with γδ T cells had a significantly lower percentage of live cells after 24 hours and 48 hours compared with vehicle-treated cells (n = 3, $p = 0.003$ and 0.0002, respectively). Significance was determined using two-way ANOVA with Bonferroni's multiple comparisons test.

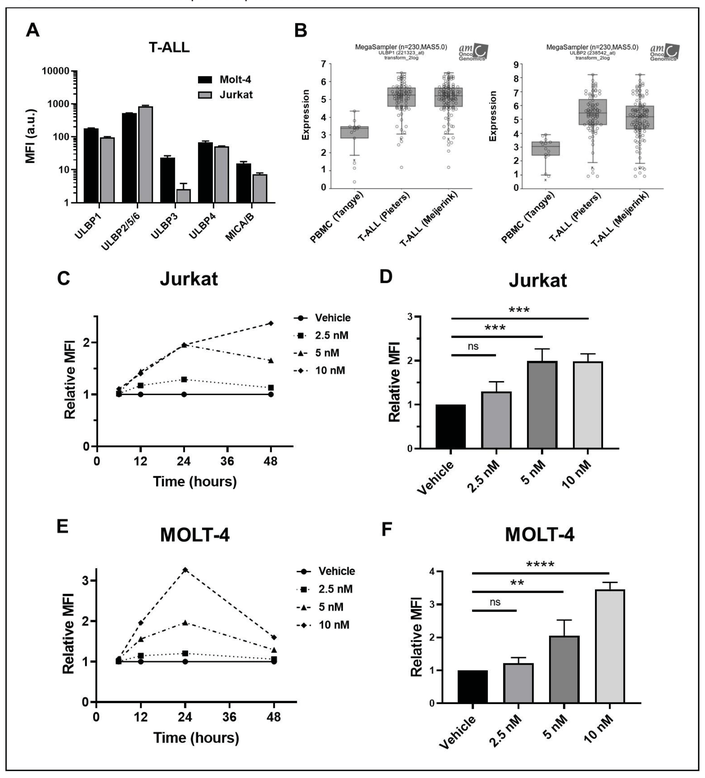

Figure 6. Bortezomib significantly increases expression of ULBP2/5/6 in T-ALL cell lines after 24 hours of treatment. (A) Baseline protein expression of NKG2D ligands on T-ALL was assessed by gating on Annexin V and 7-AAD double negative cells and analyzing surface marker expression via flow cytometry. Both T-ALL cell lines showed highest expression of ULBP2/5/6 (n = 3, 853.667 ± 51.287 MFI arbitrary units for Jurkat and 532.667 ± 4.041 MFI arbitrary units for MOLT-4). (B) RNA sequencing data from primary T-ALL patient samples showed elevated gene expression in ULBP1 and ULBP2 compared to gene expression in normal PBMCs . (C and E) T-ALL cell lines were treated with vehicle control or increasing doses of bortezomib for 48 hours. Live cells were gated on (7-AAD-/Annexin V-) and analyzed for changes in NKG2D ligand surface expression at the various time points and concentrations (average relative MFI, n= 3). (C) Surface expression of ULBP2/5/6 in Jurkat cells peaked between 24 and 48 hours at all doses. (D) There was a significant increase in ULBP2/5/6 with 5 nM and 10 nM bortezomib treatment at the 24 hour time point (n = 3, $p = 0.007$). (E) Surface expression of ULBP2/5/6 in MOLT4 cells peaked between 12 and 24 hours at all doses. (F) ULBP2/5/6 expression was also significantly increased on MOLT-4 cells at 5 nM and 10 nM bortezomib (n = 3, $p = 0.0037$ and $p < 0.0001$, respectively). Significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.

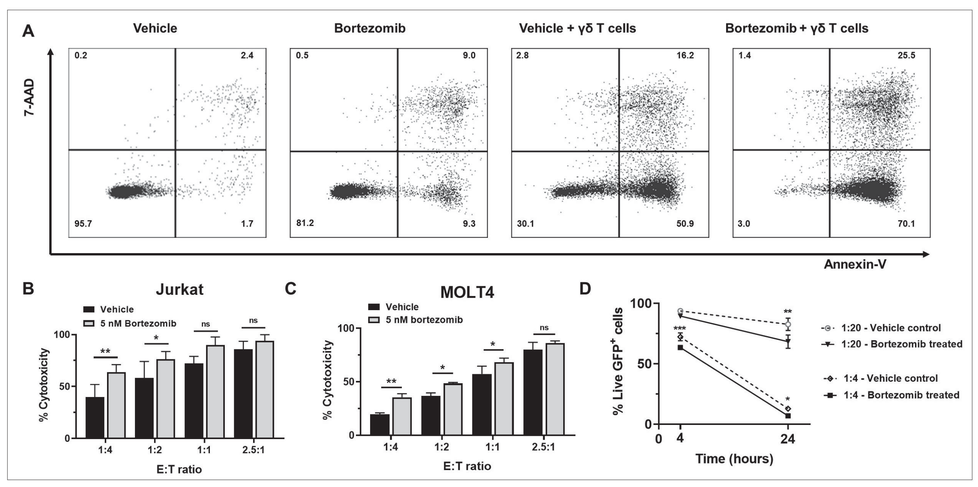

Figure 7. Bortezomib increases cytotoxicity of γδ T cells against T-ALL cell lines. (A) Representative flow plots from a 4 hour cytotoxicity assay with γδ T cells and Jurkat cells at a 1:1 effector to target (E:T) ratio. (B) Vehicle or bortezomib treated cells Jurkat and MOLT-4 cells were incubated for 4 hours with day 12 ex vivo expanded γδ T cells at 1:4, 1:2, 1:1, and 2.5:1 E:T ratios. The difference in target cell death between bortezomib-treated cells and vehicle-treated cells was compared at each E:T ratio. Replicates represent γδ T cells expanded from 3 different donors. Bortezomib-treated Jurkat cells had a significantly higher cytotoxicity (% dead target cells) compared to vehicle-treated Jurkat cells at the 1:4 and 1:2 E:T ratios (n = 3, $p = 0.0054$ and 0.0448, respectively). (C) MOLT-4 cells treated with bortezomib also had a significantly higher percentage of dead target cells at the 1:4, 1:2, and 1:1 E:T ratios (n = 3, $p = 0.001$, 0.014, and 0.023, respectively). (D) After 24 hours of treatment with bortezomib or vehicle, GFP+ Jurkat cells were incubated with day 11 ex vivo expanded γδ T cells for 4 and 24 hours at a 1:4 or 1:20 E:T ratio. The percentage of remaining live GFP+ cells (GFP+/7-AAD-) was determined at each time point to determine whether bortezomib treatment increased the cytotoxicity of GFP+ Jurkat cells compared to vehicle-treated cells. Bortezomib-treated Jurkat cells had a significantly lower percentage of live cells after 24 hours compared with vehicle-treated cells at both E:T ratios (n = 3, $p = 0.011$ and $p = 0.003$, respectively). Significance was determined using two-way ANOVA with Bonferroni's multiple comparisons test.

Conclusions

- ULBP2/5/6 surface expression significantly increases with 24 hour bortezomib treatment at 5 or 10 nM in AML and T-ALL cell lines
- Administering γδ T cells 24 hours after bortezomib treatment in humans should not negatively affect their *in vivo* cytotoxicity against cancer cells
- Bortezomib treatment significantly increased cytotoxicity of *ex vivo* expanded γδ T cells at low E:T ratios
  - Target cell death >30% was seen at 1:4 E:T ratio in all cell lines
- Bortezomib treated cells were killed faster than vehicle control treated cells in a 24-48 hour cytotoxicity assay
- Demonstrates feasibility of γδ T immunotherapy with stress antigen inducing drugs, such as bortezomib, in the AML and T-ALL setting without having to develop antigen specific immunotherapies

Future Directions

- Investigate whether bortezomib treatment increases NKG2D ligand surface expression on primary cells from AML and T-ALL patients
- Determine if bortezomib treatment enhances susceptibility of primary AML and T-ALL cells to γδ T cell-mediated cytotoxicity in an *in vitro* based cytotoxicity assay
- Perform *in vivo* experiments to investigate whether bortezomib and γδ T cells combination decreases tumor burden and/or increases survival in mice
  - Inject luciferase-expression Nomo-1 and Jurkat cells into NSG mice and treat with vehicle control, bortezomib alone, γδ T cells alone, or bortezomib + γδ T cells
  - Measure tumor burden via bioluminescence imaging and overall survival

Acknowledgements

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Development and Validation of the HIV Adolescent Readiness for Transition Scale: HARTS

Brian Zanoni,[1] Moherndran Archary,[2] Thobekile Sibaya,[2] Nicholas Musinguzi,[3] Jessica Haberer [4,5]

Emory University School of Medicine[1]; University of KwaZulu-Natal[2]; Mbarara University of Technology[3]; Massachusetts General Hospital[4]; Harvard Medical School[5]

Brian Zanoni
bzanoni@emory.edu

INTRODUCTION

Background

- Approximately 320,000 perinatally-HIV infected children and adolescents are living in South Africa.
- There are no guidelines on how or when to transition adolescents from pediatric to adult care in South Africa.
- In multiple settings, adolescents living with HIV have poor retention in care and viral suppression after transitioning to adult care.

Objective

- We designed and validated the HIV Adolescent Readiness to Transition Scale (HARTS) to determine when an adolescent is ready to transition to adult care and to identify at-risk adolescents who may need further interventions prior to transition.

METHODS

HARTS Development:

Setting: Mahatma Gandhi Hospital, KwaMashu, South Africa

Inclusion criteria:

- Perinatally HIV-infected
- Receiving ART
- Fully aware of their HIV status

Focus Groups:

1. Adolescents: 20 participants between ages 15 and 24 years
2. Healthcare Providers: 11 (physicians, nurses, counselors, social workers and pharmacists)
3. The HARTS questionnaire was revised based on participant feedback using consensus.

Psychometric Properties

- 131 adolescents receiving ART completed HARTS
- Generalized linear equation models with the overall HARTS score and with the individual domains
- Correlated the responses to self-described transition readiness and age using liner regression
- Test-retest variability with 13 (10%) adolescents
- Cronbach's alpha

HARTS Validation:

Setting: Prince Mshiyeni Hospital, Umlazi, South Africa

Inclusion criteria:

- Perinatally HIV-infected
- Receiving ART
- Fully aware of their HIV status
- Prior to transition to adult clinic

Participants:

- 199 Adolescents completed HARTS prior to transition

Analysis:

- Viral suppression (<200 copies/ml) measured after 12 months in adult clinical care
- Multivariable logistic regression based on the continuous HARTS value and covariates with a p value of less than 0.2 on bivariate analysis
- Adjusted for age at ART initiation, ART regimen, sex, disclosure status, drug and alcohol use, peer support, and self-esteem

RESULTS

Development of HARTS

Table 1: Participant characteristics for HARTS development and validation

Enrollment characteristics	Scale Development (n=131)	Scale Validation (n=199)
Recruitment site	Mahatma Gandhi Memorial Hospital	Prince Mshiyeni Hospital
Median age at enrollment (years) (IQR)	13.95 (13.15 – 14.75)	13 (12 - 13)
Median age at ART initiation (years) (IQR)	7.13 (4.73 – 8.91)	8 (5 - 9)
Female n (%)	64 (49%)	98 (49%)
First-line ART regimen n (%)	111 (85%)	184 (93%)
Drug / alcohol abuse n (%)	35 (3%)	19 (10%)
Documented Disclosure n (%)		89 (45%)
Outcomes	(n=50)	
Viral suppression after transition n (%)	39/50 (78%)	112 (57%)

- For the test-retest variability the mean score on test 1 was 36.3 (SD 7.3) and 36.9 (SD 6.7) on test 2 with no statistical difference in means (p=0.69).
- Positive correlations with self-described transition readiness were significant with the overall HARTS score (p<0.0004) and domains of health navigation (p=0.028), self-advocacy (p=0.0014), and health literacy (p=0.0023). Cronbach's alpha was 0.78.

Table 2: HARTS question correlation to individual domains and overall score

HARTS Question	Disclosure Coef; p-value (95% CI)	Health Navigation Coef; p-value (95% CI)	Self Advocacy Coef; p-value (95% CI)	Health Literacy Coef; p-value (95% CI)	Overall HARTS Coef; p-value (95% CI)
HARTS 1	0.54; <0.001 (0.33 – 0.76)				0.43; <0.001 (0.26 – 0.60)
HARTS 2	0.53; <0.001 (0.33 – 0.74)				0.38; <0.001 (0.20 – 0.56)
HARTS 4	0.32; 0.005 (0.09 – 0.54)				0.26; 0.006 (0.07 – 0.44)
HARTS 5	0.01; 0.927 (-0.21 – 0.23)				0.11; 0.249 (-0.08 – 0.31)
HARTS 6		0.63; <0.001 (0.50 – 0.76)			0.61; <0.001 (0.48 – 0.75)
HARTS 7		0.29; 0.001 (0.12 – 0.47)			0.24; 0.010 (0.06 – 0.43)
HARTS 8		0.76; <0.001 (0.64 – 0.88)			0.64; <0.001 (0.48 – 0.79)
HARTS 9		0.50; <0.001 (0.34 – 0.66)			0.59; <0.001 (0.45 – 0.73)
HARTS 3			0.44; <0.001 (0.28 – 0.60)		0.51; <0.001 (0.35 – 0.66)
HARTS 10			0.45; <0.001 (0.29 – 0.60)		0.48; <0.001 (0.32 – 0.64)
HARTS 11			0.73; <0.001 0.62 – 0.85)		0.72; <0.001 (0.59 – 0.85)
HARTS 12			0.34; <0.001 (0.17 – 0.51)		0.36; <0.001 (0.18 – 0.53)
HARTS 13			0.21; 0.032 (0.02 – 0.40)		0.30; 0.001 (0.12 – 0.48)
HARTS 14				0.34; 0.001 (0.14 – 0.53)	0.26; 0.006 (0.07 – 0.44)
HARTS 15				0.55; <0.001 (0.35 – 0.75)	0.44; <0.001 (0.27 – 0.60)
HARTS 16				0.28; 0.011 (0.06 – 0.49)	0.21; 0.031 (0.02 – 0.40)

Validation

Table 3: Multivariable logistic regression for virologic failure among non-substance using adolescents on first-line ART prior to transition to adult care

Covariate	Adjusted Odds Ratio	P-value	95% CI
HARTS Score (10 point increase)	0.44	<0.001	O.29 – 0.69
Female	2.39	0.023	1.13 – 5.10
Alcohol use	4.08	0.001	1.84 – 9.04
Disclosure	0.46	0.028	0.24 – 0.95
Age at ART initiation	1.21	0.003	1.07 – 1.38

- For substance abusing participants who were on first line ART, the HARTS score was not associated with viral failure (OR 1.58; p=0.35; 95% CI 0.61 – 4.08).
- For non-substance abusing participants who were on second line ART, the HARTS score was not associated with viral failure (OR 0.93; p=0.74; 95% CI 0.64 – 1.37).
- Age at time of transition was not significantly associated with viral suppression 12 months after transition to adult care.

CONCLUSIONS

The HARTS questionnaire is a validated scale that can be used to determine which adolescents may require additional interventions prior to transitioning to adult care to improve retention in care and viral suppression. It is particularly useful for adolescents on first line ART who are not abusing drugs to predict who may not need additional services during transition from pediatric to adult care.

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FVIII-SPECIFIC CD4 T CELL PROLIFERATION REQUIRES MULTIPLE PREVIOUS EXPOSURES TO FVIII

Patricia E. Zerra[1,2], MD, Seema R. Patel, PhD[2], W. Hunter Baldwin[2], John Healey[2], Ernest Parker[2], Courtney Cox[2], Pete Lollar, MD[2], Sean R. Stowell, MD, PhD[1] and Shannon L. Meeks, MD[2]

[1]Center for Transfusion and Cellular Therapies, Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA

[2]Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta/Emory University School of Medicine, Atlanta, GA

INTRODUCTION

FVIII replacement in hemophilia A can be complicated by the development of neutralizing anti-FVIII IgG alloantibodies (inhibitors)[1,2]. These inhibitors can actively block FVIII activity and prevent optimal replacement efficacy[3]. Despite their significant clinical implications, there are currently no prophylactic therapies to prevent inhibitor development. This is likely in part due to a poor understanding of the key immune regulators governing inhibitor formation.

In contrast to other model antigens, inhibitor formation occurs only following multiple FVIII exposures both in humans and mouse models. This suggests that early exposure events may prime subsequent development of long-lasting antibodies. In particular, as CD4 T cells facilitate IgG formation, it is possible that each FVIII exposure event may propagate a CD4 T cell response to a threshold necessary to generate an optimal IgG response. Despite previous studies suggesting that CD4 T cells play an important role in inhibitor development[4,5], their timing and overall role in this key immune response remains incompletely understood. Thus, defining the role of CD4 T cells in inhibitor development is important if novel therapeutics for inhibitor prevention are to ever be realized.

MATERIALS AND METHODS

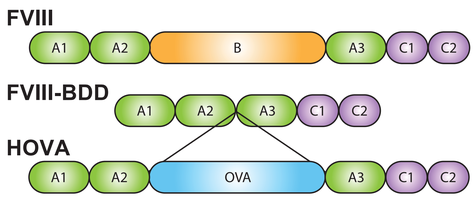


Figure 1. HOVA development. As no tools exist to study FVIII specific CD4 T cells, we engineered the model antigen, ovalbumin (OVA), into the B domain site of B-domain-deleted (BDD) FVIII for tracking antigen specific CD4 T cells. We utilized human FVIII containing an AvrII site at the end of the A2 domain and a BsiWI site in the activation peptide. Annealing of forward and reverse ultramers (containing AvrII and BsiWI sites flanking the ova consensus sequence), followed by cutting with AvrII and BsiWI which enabled us to ligate the product into human BDD FVIII.

Testing HOVA FVIII activity, immunogenicity, and function. FVIII activity of HOVA was assessed by a clotting assay. Immunogenicity was assessed by administering either HOVA or FVIII to hemophilia A mice, followed by anti-FVIII IgG measurement in plasma via ELISA. To determine OVA CD4 T cell epitope function in HOVA, CD4 T cells from OTII mice that express a T cell receptor specific to $OVA_{323-339}$ were CFSE-labeled and cultured with HOVA *in vitro* for 7 days. Proliferation was evaluated by flow cytometry.

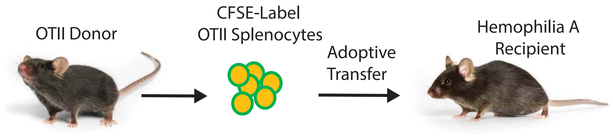


Figure 2. Adoptive Transfer of CFSE-labeled OTII splenocytes. To test whether HOVA exposure results in OTII activation *in vivo*, CFSE-labeled splenocytes from OTII transgenic mice with CD4 T cells specific for HOVA were adoptively transferred into naïve or highly immunized hemophilia A mice, followed by HOVA administration. Flow cytometric analysis assessed OTII CD4 T cell proliferation and activation.

RESULTS

HOVA is immunogenic in mice with Hemophilia A

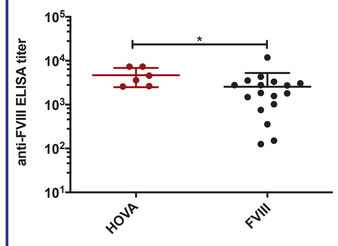


Figure 3. Anti-FVIII Antibody Titers. After 4 weekly doses of 2 µg HOVA or recombinant FVIII, plasma was evaluated for anti-FVIII IgG by ELISA. Error bars indicate SD. Horizontal lines represent mean of each group. *P=0.03, Mann-Whitney test. (HOVA also had equivalent FVIII activity via measurement of clotting time.)

HOVA CD4 T cell epitope is functional *in vitro*

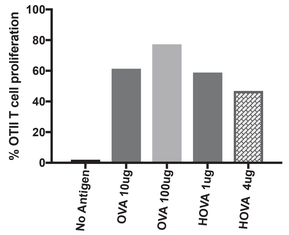


Figure 4. Splenocytes from OTII transgenic mice were CFSE-labeled, then cultured for 7 days either with no antigen, with 10 or 100 µg OVA or with 1 or 4 µg HOVA *in vitro*. OTII CD4 T cell proliferation was assessed by flow cytometry.

No FVIII-specific CD4 T cell proliferation or activation after 1-2 exposures to HOVA *in vivo*

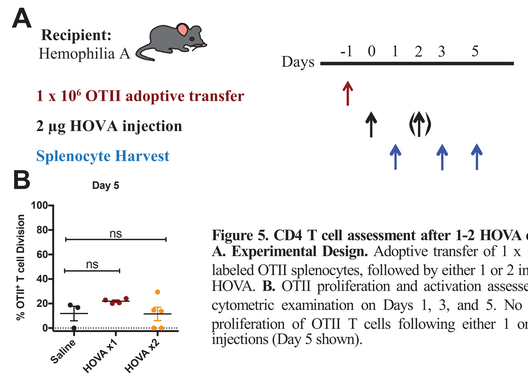


Figure 5. CD4 T cell assessment after 1-2 HOVA exposures. A. Experimental Design. Adoptive transfer of 1 x 10^6 CFSE-labeled OTII splenocytes, followed by either 1 or 2 injections of HOVA. **B.** OTII proliferation and activation assessed by flow cytometric examination on Days 1, 3, and 5. No significant proliferation of OTII T cells following either 1 or 2 HOVA injections (Day 5 shown).

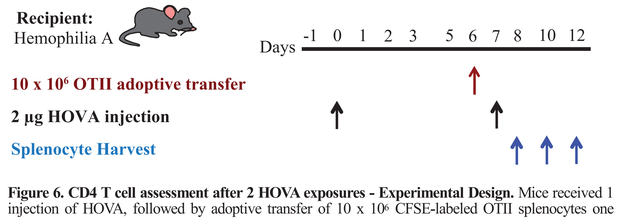


Figure 6. CD4 T cell assessment after 2 HOVA exposures - Experimental Design. Mice received 1 injection of HOVA, followed by adoptive transfer of 10 x 10^6 CFSE-labeled OTII splenocytes one week later. Mice then received a second HOVA injection on Day 7. No significant OTII T cell activation or proliferation upon analysis.

FVIII-specific CD4 T cells show proliferation and activation in highly immunized mice

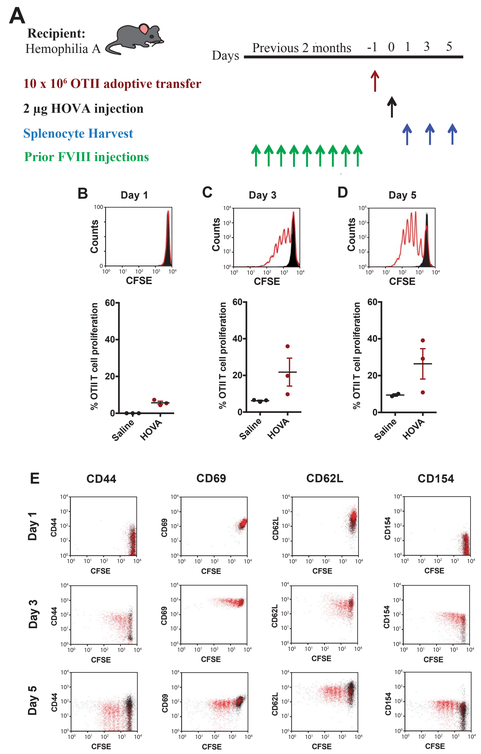
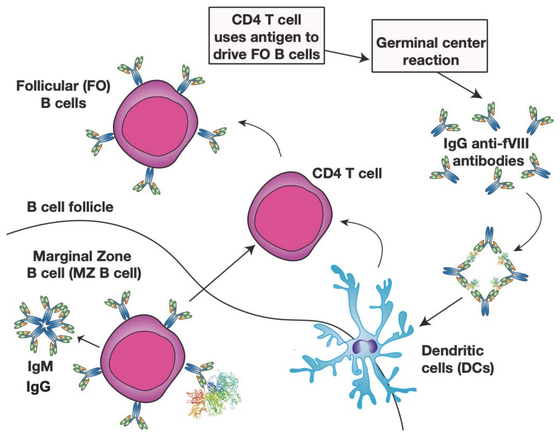


Figure 7. CD4 T cell assessment in highly immunized mice. A. Experimental Design. Mice with multiple previous exposures to FVIII were adoptively transferred with 10 million OTII splenocytes, followed by one 2 µg injection of HOVA. Representative histograms and % OTII CD4 T cell proliferation assessed on Days 1 **(B)** 3 **(C)** and 5 **(D)** by flow cytometric analysis. **E.** Representative dot plots of OTII CD4 T cell activation in mice receiving saline (black) or HOVA (red).

CONCLUSIONS



- **HOVA allows examination of the FVIII-specific CD4 T cell response**
- **FVIII-specific CD4 T cells do not proliferate in response to 1-2 doses of FVIII**
- **FVIII-specific CD4 T cell proliferation and activation requires >2 prior exposures to FVIII, consistent with the observation that multiple FVIII exposures are required prior to inhibitor development**
- **Further studies needed to elucidate the underlying mechanisms: ? Immune complex formation**

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