Weill Cornell Medicine
Pediatrics

Weill Cornell Medicine
Drukier Institute for
Children's Health

Weill Cornell Medicine Department of Pediatrics
Gale and Ira Drukier Institute for Children's Health

2nd Annual
Pediatrics Research Day

Tuesday, June 6, 2023
Belfer Research Building
2nd & 3rd Floors

Weill Cornell Medicine Pediatrics

Weill Cornell Medicine Drukier Institute for Children's Health

Pediatrics Research Day 2023 Agenda

Tuesday, June 6, 2023
Belfer Research Building 2nd and 3rd Floors

Poster Drop Off: *Friday 6/2 and Monday 6/5 10 am-5 pm, BB-1224 (Sharleen So)*
Tuesday 6/6 8 am-10:30 am, Belfer 2nd Floor, Registration Desk

Trainees Special Session

8:30 am – 8:45 am	Registration and breakfast	Belfer 3rd Fl
8:45 am – 11:05 am	**Oral Poster Presentations** Moderator: **Thanakorn Jirasevijinda, MD**	Belfer 3rd Fl
	Introductions	(*8:45 am – 8:50 am*)
	Brittany Watchmaker Sakhno, MD (Education & QI)	(*8:50 am – 8:58 am*)
	Hsuan-Yuan (Sherry) Wang (Translational)	(*8:58 am – 9:06 am*)
	Eric Wilsterman, MD (Clinical)	(*9:06 am – 9:14 am*)
	Sean Cullen, MD, PhD (Basic Science)	(*9:14 am – 9:22 am*)
	Justin Kotliar, MD (Education & QI)	(*9:22 am – 9:30 am*)
	Caitlin Williams, PhD (Translational)	(*9:30 am – 9:38 am*)
	Katherine Cunnane, BS (Clinical)	(*9:38 am – 9:46 am*)
	Claire Otero, BS (Basic Science)	(*9:46 am – 9:54 am*)
	Break	(*9:54 am – 10:09 am*)
	Charles Bergman, MD (Education & QI)	(*10:09 am – 10:17 am*)
	Lauren Robinson, MD (Translational)	(*10:17 am – 10:25 am*)
	Senayit Demie, MD (Clinical)	(*10:25 am – 10:33 am*)
	Siriruk Changrob, PhD (Basic Science)	(*10:33 am – 10:41 am*)
	Priyanka Narayan, AB (Translational)	(10:41 am – 10:57 am)
	Jade Matthews-Balcombe, B.S. (Basic Science)	(10:57 am – 11:05 am*)*
11:05 am – 11:15 am	*Break*	
11:15 am – 11:25 am	P30 Funding Opportunity **Daniel Fitzgerald, MD** **Kyu Rhee, MD, PhD**	Belfer 3rd Fl
11:25 am – 11:30 am	Closing remarks **Genevieve Fouda MD, PhD**	Belfer 3rd Fl

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***Poster Drop Off**:* *Friday 6/2 and Monday 6/5 10 am-5 pm, BB-1224 (Sharleen So)*
Tuesday 6/6 8 am-10:30 am, Belfer 2nd Floor, Registration Desk

11:00 am – 12:00 pm	Registration / Lunch	Belfer 3rd Fl
	Research Resources Exhibitor Tables Posters Open	Belfer 3rd Fl Belfer 2nd Fl
12:00 pm – 12:10 pm	Welcome Address **Virginia Pascual, MD** **Sallie Permar, MD, PhD**	Belfer 3rd Fl
12:10 pm – 12:25 pm	Research Update and Resources **Sujit Sheth, MD**	Belfer 3rd Fl
12:25 pm – 1:35 pm	**Session 1: Education, QI and Clinical** Moderator: **Adin Nelson, MD**	Belfer 3rd Fl
12:25 pm – 12:50 pm:	**Chani Traube, MD** *Beware the Aftermath: Post-Intensive Care Syndrome*	(20 min/5 min Q&A)
12:50 pm – 1:35 pm	Accepted Abstracts (10 min/5 min Q&A)	
	Rachel Arnesen, MD *Factors Influencing the Quality of Narrative Assessments of Medical Students' Clerkship Performance*	*(12:50 pm - 1:05 pm)*
	Kevin Dore, MD *Cryoprecipitate Use in Hospitalized Children: A PHIS Database Study*	*(1:05 pm - 1:20 pm)*
	Jermie Gandhi, MD, MPH *Venous Thromboembolism in Pediatric Inflammatory Bowel Disease: A Scoping Review*	*(1:20 pm - 1:35 pm)*
1:35 pm -1:45 pm	*Break*	
1:45 pm – 2:55 pm	**Session 2: Basic and Translational Sciences** Moderator: **Haiying Zhang, PhD**	Belfer 3rd Fl
1:45 pm – 2:10 pm	**Emilie Grasset, PhD** *Exploring the Role of Mesenteric Adipose Tissue in Anti-microbial Immunity*	(20 min/5 min Q&A)

2:10 pm - 2:55 pm Accepted Abstracts (10 min/5 min Q&A)

Ria Goswami, PhD *(2:10 pm - 2:25 pm)*
Gastrointestinal Commensal Bacteria Prevent Infection by Metabolizing Tryptophan and Regulating the Host Arylhydrocarbon Receptor.

Preetha Balasubramanian, PhD *(2:25 pm – 2:40 pm)*
Single Cell Analysis Unravels CD4 T Cell Heterogeneity in Systemic Lupus Erythematosus

Serena Lucotti, PhD *(2:40 pm – 2:55 pm)*
The Lung Pro-Thrombotic Niche Drives Cancer-associated Thromboembolism via Exosomal ITGB2

2:55 pm – 3:15 pm Break

3:15 pm – 4:15 pm **Keynote Address** Belfer 3rd Fl
Moderator: **David C. Lyden, MD, PhD**
Speaker: **Agata Smogorzewska, MD, PhD**
Genomic Landscape of Fanconi Anemia Cancers

4:15 pm - 5:00 PM **Career Development Panel** Belfer 3rd Fl
Moderator: **Camilia Martin, MD, MS**
Panelists: **Erika Abramson, MD, MSc**
Jennie Ono, MD
Oleh Akchurin, MD, PhD
Genevieve Fouda, MD, PhD

5:00 pm – 7:00 pm **Poster Session & Reception** Belfer 2nd Fl

6:50 pm Awards Ceremony and Closing Remarks
Virginia Pascual, MD
Sallie Permar, MD, PhD

Moderators, Presenters and Panelists

Erika Abramson, MD, MSc
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Former WCM/NYP Fellow, Pediatric Gastroenterology

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Oral Poster Abstracts

Milestones Matter: Using “Milestone Toolkits” to Improve Parental and Provider Recognition of Delays and Reduce Early Intervention (EI) Disparities

Presenting Author: Brittany Watchmaker Sakhno, MD (Resident, brw9094@nyp.org)

Authors: Lee Nancy, MD, Watchmaker Brittany, MD, Abramson Erika, MD, Osorio Nena, MD

Background: Literature suggests that initiation of appropriate services through Early Intervention (EI) is key to improved long-term developmental outcomes. Increased time to diagnosis and lower rates of EI referral disproportionately affect low socio-economic status (SES) and non-English speaking children, yet there is a paucity of literature on methods to mitigate disparities in EI referral practices. Our aim was to improve EI referral rate of eligible patients from pre-pandemic baseline (3%) to the national rate of children with at least one developmental delay (15%).

Methods: A resident-led Quality Improvement (QI) study utilized planned sequential experimentation to test a multiprong intervention “Milestones Matter” (Figure 1, Driver Diagram) on a family of measures including outcomes (parent-reported completion of EI evaluation and parental satisfaction) and processes (Milestone toolkit distribution and EI referral). The toolkit included an age-appropriate milestone card (available in English, Spanish, Bengali, Arabic) with tips for promoting developing and a developmentally appropriate item to take home (Figure 1). All eligible patients aged 0-36 months seen in a primary care clinic serving low-SES and many non-English speaking patients were included. Parental satisfaction was assessed via 7-item survey. Parent-reported anxiety regarding detection of development delays was our balancing measure. Run chart rules and Associates in Process Improvement (API) rules were applied to detect signal of change and special cause variation, while descriptive statistics was used to analyze survey data.

Results: Milestone Toolkit distribution rate was high (86%, Figure 2); 80% of parents answered “yes” to learning from the Milestone Toolkit (n = 67). EI referral rate was improved from 3 to 6% (Figure 3). Self-reported completion of EI evaluation was 74%. Of 36 referred patients post-intervention, 9 declined while 2 aged out. Out of 20 parents, only 2 reported anxiety/stress after receiving a Toolkit.

Conclusions: Referral rates to EI doubled after initiation of the Milestones Matter intervention, suggesting that this intervention may help improve parental and provider recognition of developmental delays, including in low SES and non-English speaking patients. Parental decline of EI services proves a major obstacle in timely evaluation of delays, and future efforts will focus on addressing parental hesitation.

Cytomegalovirus Pentameric Glycoprotein Complex is not required for placental transmission in a nonhuman primate model of congenital infection

Presenting Author: Hsuan-Yuan (Sherry) Wang (Graduate Student, hsw4002@med.cornell.edu)

Authors: Wang Hsuan-Yuan (Sherry), Taher Husam, Kreklywich Craig N, Streblow Daniel N, Schmidt Kimberli A, Scheef Elizabeth A, Otero Claire E, Valencia Sarah M., Gompers Andrea, Singh Parul, Iyer Smita S, Tarantal Alice F, Kaur Amitinder, Malouli Daniel, Früh Klaus, Permar Sallie R.

Congenital Cytomegalovirus (cCMV) infection is the leading infectious cause of neonatal neurologic impairment. Despite decades of study, essential virologic determinants of placental CMV transmission remain unclear. CMV pentameric glycoprotein complex (PC), composed of glycoproteins H and L (gH, gL), unique long (UL)128, UL130, and UL131a, facilitates entry into epithelial, endothelial and myeloid cells. As the major target of neutralizing antibody responses, PC is currently being evaluated as a vaccine candidate. However, the role of PC in placental CMV transmission is unclear. To address this question in an animal model recapitulating CMV infection and human pregnancy, we studied infection of pregnant rhesus macaques (RM) with rhesus CMV (RhCMV). We constructed a PC-deleted RhCMV by deleting the UL128 and UL130 homologues. As expected, PC-deleted RhCMV was unable to infect non-fibroblast cells in vitro. Dissemination and plasma viremia was also reduced compared to a PC-intact full-length (FL) virus in primary infection of RhCMV-naïve RM. When CD4+ T cell-depleted dams were inoculated intravenously with PC-deleted or FL RhCMV (n = 3, respectively) at the end of first trimester, placental transmission was observed for both viruses. Interestingly, while PC-deleted and FL RhCMV infection developed similar plasma viremia in CD4+ depleted dams, the PC-deleted group was noted with less viral shedding in urine and saliva. We next inoculated 6 immunocompetent seronegative dams with PC-deleted RhCMV and 2 of 6 dams transmitted virus (33%) to the fetus based on detection of RhCMV in amniotic fluid, similar to the rate of vertical transmission observed in seronegative dams co-infected with FL RhCMV and low passage isolate UCD52 (2 of 6 dams). As expected, plasma epithelial neutralization against the UCD52 strain was lower in the PC-deleted RhCMV-infected dams compared to that of PC-intact RhCMV-infected dams, as were antibody-dependent phagocytosis (ADCP) responses (5 of 6 PC-deleted RhCMV-infected dams). Interestingly, in assessing the plasma ADCP activity against the PC-intact and PC-deleted RhCMV virions in FL RhCMV infected dams, PC was shown to be a target of ADCP activity. Our data suggest PC-deleted RhCMV can efficiently cross the maternal-fetal interface after primary maternal infection at a rate similar to that of PC-intact RhCMV. Based on these data, we conclude that PC is dispensable for congenital infection, which raises the possibility that PC-specific antibodies might be limited in their ability to protect against cCMV transmission.

Evaluating Airway Management in Patients with Trisomy 21 in the Pediatric Intensive Care Unit

Presenting Author: Eric Wilsterman, MD (Fellow, erw9050@nyp.org)

Authors: Wilsterman EJ, Nellis ME, Panisello J, Al-Subu A, Breuer R, Dewan M, Ducharme-Crevier L, Kimura D, Krawiec C, Mallory P, Nett S, Orioles A, Owen E, Parsons S, Sanders R, Garcia-Marcinkiewicz A, Napolitano N, Shults J, Nadkarni V, Nishisaki A

Introduction: Children with Trisomy 21 (T21) are known to have anatomic and physiologic abnormalities that may complicate tracheal intubation. However, current tracheal intubation (TI) practice in critically ill children with T21 is not well described. We sought to describe TI practice in critically ill children with T21. We hypothesize the Adverse Airway Outcomes: AAOs(adverse TI associated events: TIAEs and/or peri-intubation hypoxemia: >20% decrease in SpO2) are higher in children with T21 as compared to those without T21.

Methods: Multicenter airway management quality improvement database (NEAR4KIDS) was queried. In addition, we collected patient's T21 status, cervical spine and cardiac conditions. The association between T21 status and occurrence of AAOs was evaluated using Chi2 and multivariable logistic regression adjusting for patient, provider, and practice covariates.

Results: Of the 8401 TI encounters from 15 pediatric general/cardiac ICUs between 2014-2020, 274 (3.3%) were TIs in patients with T21. Among those with T21, 84% had congenital heart disease, and 4% had atlantoaxial instability with cervical spine protection used in 6%. Patients with T21 were smaller (median (IQR) weight 7.8 (4.5-14.7) kg vs 10.6 (5.2-25) kg, p<0.001). Patients with T21 (vs without) were more commonly urgently intubated for oxygenation (46% vs 32%, p< 0.001)and ventilation failure (41% vs 35%, p=0.04) as opposed to elective procedural intubations. Patients with T21 had more difficult airway features (35% vs 25%, p=0.001), including upper airway obstruction (14% vs 8%, p=0.001). Patients withT21 were more likely to receive atropine (34% vs 26%, p=0.004) and less likely intubated with video laryngoscopy (30% vs37%, p=0.023). AAOs occurred similarly between those with T21 vs without (26% vs. 24%, p=0.669); adverse TIAEs occurred less in T21 (9% vs 14%, p=0.02), but hypoxemia occurred more frequently (23% vs 18%, p=0.046). After adjusting for age, indication, provider, device, paralytic, and apneic oxygenation, T21 condition was not associated with increased AAOs (OR 0.91, 95% CI 0.69-1.21, p=0.53).

Conclusions: T21 condition was not associated with higher adverse airway outcomes. Patients with T21 were intubated more frequently for respiratory failure and with atropine and less frequently with video laryngoscopy.

The Role of Khdc3 in Epigenetic Inheritance of Obesity and Metabolic Disease

Presenting Author: Sean Cullen, MD, PhD (Fellow, smc9046@nyp.org)

Authors: Cullen, Sean M, Senaldi, Liana, Smith-Raska, Matthew

Introduction: Childhood obesity and metabolic disease is an ongoing public health epidemic. There is strong evidence that inherited factors contribute strongly to disease risk, but DNA-focused approaches have failed to explain much of this inheritance. Khdc3 is a predicted critical regulator of epigenetic inheritance. We hypothesize that Khdc3 is essential for integrating environmental signals, such as exposure to a high fat diet (HFD), into dynamic heritable changes in germ cell small RNAs & DNA methylation, with important implications for the intergenerational inheritance of disease risk based on ancestral diet.

Objective: To test how Khdc3 regulates murine sperm small RNAs & DNA methylation in response to a HFD and the resultant disease risk in non-exposed descendants.

Methods: Khdc3-null (KO) and wild type (WT) male mice were fed either high fat diet (HFD) or control diet (CD) for 8 weeks. Weight gain was monitored, along with various metabolic tests. Males were then sacrificed to isolate sperm small RNA and genomic DNA. In order to elucidate the inheritance of metabolic disease, WT mice descended from Khdc3 mutant ancestors were generated. “Wild type star (WT*)” mice developed from KO grandfathers mated with WT grandmothers (F0). WT* male mice (F2) are differentiated from genetically identical WT mice derived from WT ancestors. The WT* mice will be compared to WT mice for weight gain and glucose regulation in response to a HFD, as well as comparing their sperm small RNA populations and DNA methylation patterns to both their KO ancestors and WT controls.

Results: Mice on the HFD gained significantly more weight than their control diet counterparts, as expected. Interestingly, KO mice on a HFD weighed significantly more than WT mice on the HFD at the end of the experiment. KO mice on the control diet also appear to have significantly higher levels of fasting serum glucose compared to WT mice. Sperm small RNAs of KO mice on both the CD and HFD show significant dysregulation from multiple different classes including miRNA, piRNAs, and tRNA fragments. Mir135a-1 was found to be significantly upregulated in KO mice on both a HFD and CD; this miRNA has been associated with regulating lipid and glucose metabolism. Additionally, there are a significant number of differentially methylated regions (DMR) in KO and WT sperm genomic DNA, including genes involved in small RNA regulation, such as Dicer and Piwli1. Furthermore, metabolic somatic tissues (liver, pancreas, adipose tissue) of wild type mice descended from Khdc3 mutant ancestors display evidence of metabolic dysregulation.

Conclusion: Khdc3-null (KO) mice have a baseline metabolic phenotype, predisposing to abnormal weight patterns and glucose dysregulation. Along with observed small RNA & DNA methylation sperm alterations, we believe future experiments will elucidate populations that can perpetuate these epigenetic and phenotypic changes, even those genetically wild type.

Sixty Golden Minutes: A Golden Hour Bundle for High-Risk Preterm Infants

Presenting Author: Justin Kotliar, MD (Fellow, jak9178@nyp.org)

Author: Kotliar, Justin MD, Tiwari, Priyanka MD, Davydova, Yafa MD, Lubor, Brienne MD, Karotseris, Christine MSN BSN, Taikina, Jane BSN, Connolly, Cailinn BSN, Farra, Elizabeth MSN, Milless, Laura MSN, Yap, Vivien MD, Blatt, Lauren MD

Introduction: The “Golden Hour” (GH) refers to the first sixty minutes after birth in neonates. In preterm infants, practice of evidence-based interventions during this GH has been associated with reduction in infant mortality and morbidities, including hypothermia, hypoglycemia, intraventricular hemorrhage, and bronchopulmonary dysplasia.

Objectives: The primary aim was to increase overall adherence to the elements of the GH bundle to 50% by July 2023. Secondary aims were to: 1) reduce the rates of moderate/severe hypothermia (temperature < 36°C) by 25%, 2) reduce time to antibiotic administration by 25% and 3) increase the initiation of intravenous fluid (IVF) within the GH by 25% by July 2023.

Methods: A multidisciplinary quality improvement (QI) team performed sequential interventions in inborn newborns < 32 weeks or ≤1500 grams in a level IV NICU to reduce severe hypothermia, time to antibiotics and IVF administration (outcome measures). Our process measure was adherence to the GH standardized bundle. Baseline data was collected from January 2020 to November 2021. Balance measures included hyperthermia and admission temperature in preterm newborns that did not meet the GH inclusion criteria. Statistical process control charts and established Associates in Process Improvement rules for special cause variation were used to display and analyze data.

Results: A total of 255 infants were included (163 pre-GH bundle, 92 post-GH bundle) through three PDSA cycles. Mean gestational age and birth weight pre/post-GH bundle were 29 3/7 weeks, 1189 grams and 29 5/7 weeks, 1226 grams respectively (P-values 0.3, 0.42). The adherence to the GH bundle was 86%. In regard to outcome measures, the mean time to antibiotic administration decreased from 132 minutes to 78 minutes, the percentage of infants receiving IVF within the GH increased from 30% to 71%, and the rates of moderate/severe hypothermia decreased from 25% to 4%. For balance measures, there were no cases of hyperthermia or increased rates of hypothermia in excluded preterm newborns.

Conclusion: A multidisciplinary QI team successfully and safely demonstrated adherence to the GH bundle and subsequently an improvement in time to antibiotic administration, IVF initiation, and moderate/severe hypothermia. Future plans include incorporating the GH interventions as part of a larger bronchopulmonary dysplasia prevention bundle QI initiative.

SARS-CoV-2 mRNA-LNP vaccination elicits broadly binding IgG responses in young children.

Presenting Author: Caitlin Williams, PhD (Postdoc, caw4009@med.cornell.edu)

Authors: Williams, C.A., Herbeck, S., Kelly, M.S., Hurst, J., Rotta, A.T., Turner, N.A., Dalapati, T., Pulido, N., Aquino, J.N., Lugo, D.J., Pfeiffer, T.S., Rodriguez, J., Burke, T.W., McClain, M.T., Woods, C.W., Fouda, G., Permar, S.R.

Introduction: The Food and Drug Administration recently approved use of two SARS-CoV-2 mRNA-LNP vaccines for the prevention of severe COVID-19 in children less than 5 years of age. These vaccines feature a 2 dose (Moderna: mRNA-1273) or 3 dose (Pfizer: BNT162b2) monovalent primary series. Currently, both manufacturers recommend a heterologous prime-boost method of the wild-type spike protein as the primary immunogen and the omicron BA.4/BA.5 spike as the heterologous boost immunogen.

Objectives: To characterize vaccine-elicited spike-specific IgG responses in children less than 5 years of age.

Methods: 77 children aged 6 months to 4 years of age were enrolled in the Biospecimens from RespirAtory Virus Exposed Kids (BRAVE) Study at Duke University. Forty children were immunized with two doses of the Moderna (mRNA-1273) vaccine and 37 were immunized with three doses of the Pfizer-BioNTech (BNT162b2) vaccine. Participants were recruited prior to the current schedule which includes a bivalent third dose. Serum samples were collected 1 month after completion of the primary series. Serum samples were diluted 1:1000 for binding antibody multiplex assays (BAMA) to assess IgG binding to 11 spike proteins. Here we report the background corrected mean fluorescence intensity (MFI) of IgG bound to individual Spike proteins.

Results: Children immunized with either vaccine developed robust IgG responses with binding to spike proteins of variants of interest as well as omicron subvariants BA.1.1, BA.2, and BA.4/5. The median Omicron (B.1.1.529)-specific IgG MFI was 7618 in the mRNA-1273 group and 7660 in the BNT162b2 vaccine group (p>0.05). The median D614G MFI was 18561 in the mRNA-1273 group and 13847 in the BNT162b2 vaccine group (p=0.0233). When stratified by age, there was no statistically significant difference between spike binding antibody responses among infants (<1 year), toddlers (1-3 years), and young children (4 years).

Conclusions: We demonstrate that mRNA-LNP vaccines used to prevent severe COVID-19 disease in older children and adults elicit robust spike-specific IgG as well as IgG binding breadth in children under the age of 5 years. We are among the first to report on vaccine elicited Omicron specific IgG responses in children under the age of 5. Recent studies of the inactivated CoronaVac vaccine have demonstrated 51% efficacy in preventing omicron infection in children aged 2-5. Here we demonstrate that the monovalent mRNA-LNP vaccines generate broadly binding IgG responses capable of binding to the highly mutated omicron strains, which has been previously unreported. Further investigation into omicron specific functional responses such as neutralization are warranted; however, these findings indicate that vaccination of young children will be an effective strategy to prevent severe COVID-19 in young children.

SYNGAP1-Related Developmental and Epileptic Encephalopathy: Meaningful Clinical Outcomes and Development of a Disease Concept Model

Presenting Author: Katharine Cunnane, BS (Medical Student, kcu4001@med.cornell.edu)

Authors: Cunnane Katharine A, Grinspan Zachary M, Cabassa Miskimen Andrea, Arnesen Rachel

SYNGAP1 is a heterogeneous genetic disorder that commonly gives rise to intellectual disability in association with epileptic seizures beginning at 2 years of age. Other common features of SYNGAP1 include behavioral disturbances with high rates of autism, delayed or absent language, impaired motor skills, gastrointestinal issues, and sleeping difficulties. Though much work has been done in the past decade to characterize the features of SYNGAP1, no disease-modifying treatments exist. Treatment is limited to symptom management, requiring caregivers to seek help from several specialists, administer multiple medications, and assume time and cost burdens. This study aims to create a “disease concept model,” which maps out the features of SYNGAP1 that have the largest impact on the lives of patients and their caregivers. We report the most challenging features of SYNGAP1 from the perspective of both caregivers and physicians to guide treatment and identify meaningful outcomes to be evaluated in future clinical trials. This study used a variety of qualitative research modalities, including a literature review of SYNGAP1, semi-structured interviews with caregivers, and a caregiver survey. Supplemental expert interviews and chart review using the Weill Cornell Electronic Health Record are currently being done to evaluate the most salient features of SYNGAP1 according to providers. The literature review and caregiver interviews indicate that the most challenging aspects of SYNGAP1 include difficult behaviors and language impairments. The caregiver survey is in progress, and it seeks to validate and further characterize these identified features. Once all data is obtained, we will identify discrepancies between the priorities of caregivers and providers in treating SYNGAP1. We will then recommend a set of instruments that quantitatively measure the most challenging, prevalent features of SYNGAP1 according to the disease concept model for use in future clinical trials and clinical practice.

Rhesus macaque CMV viral Fcγ receptors are not required for placental transmission but aid in persistence of viremia in vivo

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Cytomegalovirus (CMV) is the most common congenital infection and a leading cause of neurologic birth defects. Thus, CMV is a top priority for vaccine development. Evidence suggests that CMV-specific Fc-mediated antibody effector responses, such as antibody dependent cellular phagocytosis and cytotoxicity, are key for prevention of congenital CMV. However, human CMV expresses three Fcγ receptors that have been shown in vitro to bind IgG and synergistically interfere with anti-CMV IgG engagement of host Fcγ receptors (FcγRs). We recently identified three functionally homologous viral Fcγ receptors (vFcγRs) in rhesus macaque (RM) CMV (RhCMV), which allow us to validate the function of vFcγRs in vivo. Using a full-length (FL) RhCMV backbone, we produced a mutant RhCMV lacking all identified vFcγRs (ΔvFcγR). CMV-seronegative male RMs infected with ΔvFcγR (n=4) and FL (n=3) RhCMV experienced similar peak DNAemia levels 1 week post infection but ΔvFcγR RhCMV DNAemia was contained more quickly. This control of DNAemia closely followed the development of RhCMV-specific IgG responses which have similar kinetics post infection in both groups, with peak antibody titers 3–4 weeks after infection. This pattern suggests that anti-RhCMV antibody-mediated immunity may have been more effective against the RhCMV deleted of vFcγRs than the RhCMV FL virus. We then investigated whether the vFcγRs are needed for vertical transmission of RhCMV by infecting rhesus dams in the late first/early second trimester with either ΔvFcγR (n=3) or FL (n=3) RhCMV following CD4+ T cell depletion, which leads to a delay in humoral immune responses and consistent congenital CMV transmission. RhCMV DNA was detected in the amniotic fluid of 2/3 ΔvFcγR RhCMV- and 3/3 FL RhCMV-infected dams, suggesting that deletion of vFcγRs impairs but does not eliminate RhCMV's ability to cross the placenta. These initial in vivo studies suggest that CMV vFcγRs are compelling targets for novel vaccines or therapeutics for prevention of congenital CMV.

A Simulation-Based Discharge Education Program (SDP) for Caregivers of Children with Tracheostomies: A Mixed Method Study on Patient Outcomes and Parental Preparedness

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Introduction: Congenital cytomegalovirus (cCMV) is the most common infectious cause of birth defects worldwide, affecting 0.5-2% of live births. The risk of cCMV infection is higher after maternal primary infection compared to re-infection or reactivation of latent virus during pregnancy, suggesting there is a partially protective role of adaptive maternal immunity against transmission. Pregnant people living with HIV/HCMV have an elevated risk (2-7%) of vertical CMV transmission. This increased risk in the setting of untreated HIV infection may be due to HIV-associated factors such as impaired CD4+ T cells, but immune correlates of transmission in HIV-infected pregnancies, and whether they are distinct to those without HIV, remain unclear.

Objectives: We aim to define the humoral immune correlates of protection against cCMV in the high-incidence setting of maternal HIV/HCMV co-infection, which will help address the gap in knowledge of protective immunologic responses against cCMV, a gap that is currently impeding HCMV vaccine development for this important demographic in the global HCMV epidemic. Specifically, we hypothesize that the magnitude, specificity, function, and placental transfer of maternal antibodies are associated with protection against cCMV transmission in HIV and CMV-seropositive pregnant people.

Methods: Using samples from 3 historical cohorts, we performed PCR on HIV/CMV-coinfected maternal plasma drawn near delivery and infant plasma collected at <2 weeks of age. We identified 15 cases of cCMV transmission. Cases were matched 1:2 by propensity score to HIV/CMV coinfected non-transmitting dyads (n=30) based on maternal age, gravida, parity, and CD4 counts. We measured total and virus-specific antibody magnitude by ELISA and Binding Antibody Multiplex Assay (BAMA). We assessed functional antibody responses by Antibody-Dependent Cellular Phagocytosis (ADCP), Antibody-Dependent Cellular Cytotoxicity (ADCC), Fc gamma receptor binding, and neutralization assays. Mann-Whitney U tests were applied to assess differences in total and functional HCMV-specific antibody responses, using p-values of <0.05 as the significance threshold for our hypothesis-generating study.

Results/Conclusions: We observed a trend of increased HCMV-specific antibody responses, including higher virion glycoprotein specific IgG concentrations and neutralizing antibody titers, in transmitting compared to non-transmitting maternal plasma. These results suggest that a high quantity of HCMV-specific antibodies is not independently protective against cCMV acquisition. In previous literature, increased ADCP and ADCC responses have been established as protective against cCMV acquisition in non-HIV infected cohorts. In this cohort, we observed similar levels of ADCC and ADCP responses in transmitting and non-transmitting groups, underscoring the variation in immune correlates in distinct maternal demographic groups and HCMV exposure status.

These results suggest that high HCMV glycoprotein-specific IgG binding and neutralizing titers could be used as a predictive marker of the risk of cCMV infection in the setting of maternal HIV/HCMV co-infection. Ongoing work will determine if the observed higher antibody responses in transmitting mothers are associated with HCMV viremia to determine whether decreasing maternal HCMV viral loads via vaccination can be a protective intervention against cCMV transmission.

Characterization of the Anti-Erythrocyte Response in Childhood Onset Systemic Lupus Erythematosus

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Introduction: Systemic Lupus Erythematosus (SLE) is a heterogenous, complex autoimmune disease. Identification of disease subsets is crucial to understanding disease pathogenesis and ultimately to the development of targeted therapies. It is widely known that SLE patients often have Coombs positivity, suggesting the presence of anti-erythrocyte antibodies. The presence of these antibodies in the absence of hemolytic anemia have long been thought to be clinically insignificant, though an association with a more severe disease phenotype has been reported [1]. We have previously demonstrated that 75% of pediatric SLE patients in our cohort have significant IgG reactivity to several erythrocyte membrane proteins [2]. Despite the high prevalence of these antibodies, little is known about the functional properties of these antibodies and the potential immunologic consequences of this anti-erythrocyte response in SLE.

Objectives: The primary objective of this project is to characterize the anti-erythrocyte response in patients with pediatric SLE.

Methods: An in-vitro erythrophagocytosis assay was used to determine the opsonizing capacity of patient serum for erythrocytes. In this assay, fluorescently labeled blood type O healthy donor erythrocytes were treated with either SLE serum or healthy control serum, and subsequent erythrocyte phagocytosis by monocytes was detected using flow cytometry. To screen for evidence of erythrophagocytosis ex-vivo, a flow cytometry panel including monocyte surface markers and intracellular staining for the erythrocyte membrane protein glycophorin A was developed and validated.

Results/Conclusions: The in-vitro erythrophagocytosis assay confirmed that in a significant subset of pediatric SLE patients (~30%), the anti-erythrocyte IgG fraction induced opsonization within blood monocytes, an activity that was absent in pediatric healthy controls. Screening of pediatric SLE patient PBMCs revealed evidence of ex-vivo erythrophagocytosis within blood monocytes, most prominently in the patients with opsonizing serum. Neither the presence of opsonizing serum nor ex-vivo erythrophagocytosis were associated with markers of anemia. These findings support the hypothesis that in a subset of patients with pediatric SLE, the presence of anti-erythrocyte membrane IgG is associated with erythrocyte opsonization and phagocytosis by monocytes in the absence of anemia. Future studies will focus on how the anti-erythrocyte response behaves longitudinally, and if there is a role for use of these assays as disease biomarkers. Our ultimate goal is to determine the role the anti-erythrocyte response may play in disease pathogenesis.

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Impact of Minority Race/Ethnicity, Language, and Public Payor Status on Pediatric Adverse Events

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Background: The intersection of equity and safety is an emerging area of study. Studies of adult patients have shown that persons minoritized due to race/ethnicity, preferred language, and public payor status are more likely to experience harm while hospitalized. This area has been underexplored in pediatrics.

Objective: To examine the association between demographic factors (patient age, race/ethnicity, preferred language, and insurance type) and adverse events (AEs) among hospitalized pediatric patients.

Methods: This retrospective observational study analyzed AE reports at two tertiary children's hospitals between 2020 to 2022. All patients 18 years old or younger and admitted to the NICU, PICU, or inpatient units were included. The hospital's electronic medical record was used to obtain patient age, race/ethnicity, preferred language, and insurance type. The hospital's voluntary AE reporting system was utilized to collect data on inpatient AEs including central line associated blood stream infections, catheter associated urinary tract infections, falls, pressure injuries, Clostridium difficile infections, adverse drug reactions, medication errors, and unplanned extubations. Additional chart review was completed for patients with a reported AE using a previously validated pediatric medical complexity algorithm. Descriptive statistics and multivariable logistic regression were used to evaluate the independent effect of patient demographics on AE occurrence.

Results: There were 17,862 hospital encounters and 373 reported AEs. Of note, 291 (78%) of all reported AEs were experienced by children with complex chronic disease and 301 (81%) of all reported AEs were medication errors. In the multivariable logistic regression, patients with public insurance, when compared to private, had greater odds of experiencing an AE (OR: 1.27, 95% CI: 1.01, 1.61), adjusting for age, race/ethnicity, and preferred language. There was no significant difference found when comparing preferred language, non-English vs English, adjusting for age, race/ethnicity, and insurance type. The association between subtypes of AEs and demographics were not analyzed given sample size limitations.

Conclusion: Public insurance was associated with higher rates of AEs in hospitalized children even after adjusting for age, race/ethnicity, and preferred language. This indicates a possible association between low socioeconomic status and risk for experiencing harm during medical care. Further research is needed to identify and mitigate the underlying structural factors leading to this health inequity.

Broadly neutralizing antibodies targeting distinct epitopes on spike protect against human betacoronaviruses

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Prior to the COVID-19 pandemic, humans were repeatedly exposed to common cold coronaviruses (CCCs) throughout their lifetime. Understanding of the immunological memory response to CCCs in SARS-CoV-2 infected individuals is required to determine whether pre-existing CCCs or de novo responses to SARS-CoV-2 can shape protective immunity against COVID-19 disease. In this study, we investigate the role of immunological imprinting on COVID-19 patients with a range of infection severity, at the serological and single B cell level. We found a strong back-boosting effect to conserved regions on subunit 2 (S2) domain on spike proteins. A majority of SARS-CoV2 infected individuals exhibit dominant serum cross-reactive antibody responses targeting beta-CCC spike and alpha-CCC nucleocapsid protein. We used single-cell sequencing to profile human CoV-reactive B cells with oligo-tagged antigen baits approach. A large proportion of CCCs-reactive antibody-secreting cells were predominantly induced at early phase of SARS-CoV2 infection, while SARS-CoV2 spike-specific B cells were enriched in the memory compartment with the substantial proportions of de novo response increased over time following recovery from infection. We characterize monoclonal antibodies (mAbs) targeting receptor-binding domain (RBD) that were derived from the de novo response to SARS-CoV2, as well as cross-reactive antibodies targeting S2 that were derived from pre-existing memory B cells. We found that the majority of the mAbs elicited during early SARS-CoV-2 infection had stronger affinity to cross-reactive CCCs antigens, indicating recall of memory B cells from previous exposures. While the majority of these antibodies were non-neutralizing, mAbs targeting the RBD of SARS-CoV-2 exhibited potent neutralizing activity. Unfortunately, the Omicron variant has limited the ability of these mAbs to maintain their neutralizing activity. Finally, we identified a broadly neutralizing mAb targeting RBD and a pan-betaCoV neutralizing mAb targeting S2, which could provide new opportunities for antibody-based interventions and key insights for developing pan-betaCoV vaccines.

Increased Sclerostin Expression in Endothelial Cells Promotes Multiple Trisomy 21 Phenotypes

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Trisomy 21 (T21), a prevalent human genetic syndrome occurring in 1:800 births, is characterized by cardiac and extra-cardiac phenotypes. Despite a definitive genetic cause, the molecular mechanisms by which T21 perturbs the development and maintenance of human tissues remains poorly defined. Using RNA-sequencing of CHD tissues from 49 T21 and 226 euploid (eCHD) patients we compared genome-wide transcript expression. Single-nuclei RNA-sequencing identified cardiac cell lineages with mis-expressed transcripts that we confirmed using RNA in situ hybridization. T21 compared to eCHD hearts had increased expression of chr21 genes and 11-fold greater levels (p=1.2E-8) of SOST (chr17), encoding the Wnt-inhibitor sclerostin, and its transcriptional activator ZNF467 (chr7), which were co-expressed in cardiac cells (P=1.5E-7). T21 endothelial cells had 12-fold more SOST RNA than euploid endothelial cells (p=2.7E-27), with downregulation of multiple Wnt pathway genes. Within the chr21 CHD critical region, the expression of DSCAM (Down syndrome cell adhesion molecule) was significantly correlated with levels of SOST (p=1.9E-5) and ZNF467 (p=2.9E-4). Wnt signaling is critical for atrioventricular canal, bone development and mass, and pulmonary vascular homeostasis. We suggest that T21-mediated increases in sclerostin inhibit physiologic Wnt activities promoting Down syndrome phenotypes. These findings imply therapeutic potential of anti-sclerostin antibodies for multiple T21 phenotypes.

Iron Sequestration in Chronic Kidney Disease

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Anemia is a common manifestation of chronic kidney disease (CKD), frequently associated with hypoferremia (depletion of the circulating iron pool). Oral iron is the recommended first-line therapy although treatment resistance is highly prevalent. Hepcidin induction in CKD presumably limits iron absorption and enhances iron sequestration within reticuloendothelial system. However, the relative contribution of iron sequestration vs. malabsorption to hypoferremia and anemia in CKD remains unclear, which precludes development of more effective treatment strategies.

To test whether iron is being sequestered in untreated CKD, we induced CKD in 8-week-old C57BL/6 mice using the 0.2% adenine diet with physiologic iron content for 8 weeks. Control mice received an identical diet without adenine. To assess effects of iron therapy, separate mouse groups received adenine diet enriched with 0.25% and 0.5% carbonyl iron. Iron content was assessed in the bone marrow, spleen, and liver by Perls stain, colorimetric-based method, and by magnetic resonance imaging (MRI).

Untreated CKD mice displayed anemia and hypoferremia, which was alleviated by the 0.5% but not by 0.25% carbonyl iron diet. Hepcidin was elevated in untreated CKD mice compared to controls, and further induced by iron therapy. Compared to controls, untreated CKD mice had a higher count of iron-positive cells in the bone marrow (39.4 ± 12.78 per high power field vs. 1.4 ± 1.14, p<0.001) and spleen (178.1 ± 39.2 vs. 54.0 ± 26.4, p<0.001), and higher liver iron content (71.6 ± 39.9 µg/g tissue vs. 42.4 ± 17.4, p=0.01). These differences remained significant after normalization for body weight. Iron therapy resulted in further iron accumulation in tissues. When assessed by MRI, 0.5% carbonyl iron resulted in a 3.6-fold increase in liver iron content, compared to untreated CKD mice (p<0.05).

Despite putative iron malabsorption due to hepcidin induction, CKD induced iron sequestration in tissues, which was further aggravated by iron therapy. This provides novel insight into the pathogenesis of the "functional iron deficiency" in CKD.

Oral Presentation Abstracts

Factors Influencing the Quality of Narrative Assessments of Medical Students' Clerkship Performance

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Background: Narrative comments on clinical assessments of students guide their development and impact residency selection. Previous studies have shown that narrative comments rarely describe students' skills or offer actionable feedback though, and factors affecting comments' quality have not been identified.

Objective: Identify factors associated with narrative comment quality on student assessments.

Methods: We examined free-text comments on 2,950 clinical assessments for medical students on the pediatrics clerkship between 2017-2021 and rated their quality using the Feedback Evaluation Tool (FET) (Ross, et al. 2013). The FET evaluates narrative comments to generate an overall quality score. We then used ANOVA tests to check what demographic or contextual factors influenced FET scores, and Pearson's correlation to test associations between FET scores and the numerical rating of student performance and word count of individual narrative comments.

Results: The mean FET score for the narrative comments was 3.42 out of 5. The FET score did not differ by student gender ($p = 0.8$) or assessor gender ($p = 0.5$). Additionally, there was no significant interaction between student and assessor gender ($p = 0.8$). Assessors' level of training significantly affected FET scores (interns: 3.53, residents: 3.34, and attendings: 3.39; p <0.01). Clinical setting also affected FET scores (inpatient: 3.45, outpatient: 3.53, ED: 3.33, and nursery: 3.13; p < 0.01). The factor associated with the largest discrepancy in FET scores was the clerkship site as the mean FET score at two community hospitals was 3.05 and 3.57, while the mean FET score at the academic center site was 3.52 (p <0.01). Higher FET score was associated with higher word count in the comments ($R2 = 0.35$, p < 0.01), but not with the numerical ratings of student performance ($R2 = 0.0002$, $p = 0.4$).

Conclusions: The quality of narrative comments on clerkship students' clinical assessments are influenced by assessors' level of training, clinical setting, clerkship site, and narrative comment word count. These differences may be explained by the extent of student-assessor interaction and by assessors' backgrounds, as the majority of residents at one of the community hospital sites were international medical graduates. Our results suggest that more training on writing narrative comments is required for assessors of all levels of training. Additionally, implementing word count requirements for narrative comments may improve their quality.

Cryoprecipitate Use in Hospitalized Children: A PHIS Database Study

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Background: Cryoprecipitate is often used to prevent and treat bleeding in the setting of surgery, trauma, liver failure, disseminated intravascular coagulopathy, and cancer. Despite its importance, little is known about the overall use and epidemiology of cryoprecipitate in pediatric populations.

Objective: To describe the prevalence and epidemiology of cryoprecipitate transfusions among hospitalized children in the United States from 2010-2019.

Design/Methods: We conducted a retrospective cohort study of hospitalized patients using data obtained from the Pediatric Health Information System (PHIS) database. Pediatric patient encounters (ages 0-18 years of age) receiving at least one cryoprecipitate transfusion from January 1, 2010, to December 31, 2019, were included. Associated data regarding demographics, diagnoses, complex chronic conditions, procedures during hospitalization, thrombosis, infection, and outcomes were extracted for eligible encounters.

Results: There were 6,284,264 total hospital encounters from 47 hospitals within the PHIS database. Of those encounters, 76,614 children received at least one cryoprecipitate transfusion, resulting in a prevalence of 1.23% (95% CI 1.22-1.24). The proportion of hospitalizations requiring a least one cryoprecipitate transfusion was constant over the decade studied (p=0.59). Fifty-six percent of the transfused cohort was male with a mean (SD) age of 2.5 (4.7). Eighty-eight percent of transfused children had a complex chronic condition and the most common diagnoses included diseases of the circulatory system (42%) and newborn encounters (31%). After adjusting for age, ECMO support, mechanical ventilation (except for infection), and the need for surgical intervention, every additional cryoprecipitate transfusion increased the odds of thrombosis (1.09, 95% CI: 1.08, 1.11), infection (1.08, 95% CI: 1.05, 1.10), and mortality (1.30, 95% CI: 1.28, 1.32).

Conclusions: Despite efforts to promote more restrictive transfusion practices, the prevalence of cryoprecipitate use has remained stable in hospitalized children in the United States over the past decade. Prospective interventional trials should focus on those children undergoing cardiopulmonary bypass surgery and neonates to investigate the safety and efficacy of cryoprecipitate in children.

Venous Thromboembolism in Pediatric Inflammatory Bowel Disease: A Scoping Review

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Background: Inflammatory Bowel Disease (IBD) is defined by chronic inflammation of the gastrointestinal tract and includes Crohn's disease and ulcerative colitis. GI complications include rectal bleeding and diarrhea. In addition, extra-intestinal manifestations are common, and one of the most serious complications is venous thromboembolism (VTE), including cerebral venous sinus thrombosis (CVST). While there are prevention and treatment guidelines for VTE in adult IBD patients, no consensus exists for pediatric patients. In this scoping review, we sought to determine if there is sufficient data to establish VTE-related guidelines in pediatric IBD patients. While it is known that rates of VTE are increased in both adult and pediatric patients, there is limited data on risk factors in these children and there are no guidelines for the prevention or management of VTE in children with IBD.

Objectives: To evaluate the available literature describing VTE risk factors and outcomes in children with IBD. In this scoping review, we sought to determine if there is sufficient data to establish VTE-related guidelines in pediatric IBD patients.

Methods: Detailed literature searches were completed using Ovid MEDLINE ALL (1946-2021), Ovid Embase (1974-2021), and the Cochrane Library. PRISMA guidelines were followed. Search terms and keywords were included for both IBD and VTE. Study abstracts were screened by 2 reviewers, with discrepancies resolved by consensus. Full text review followed the same methods. The Downs and Black checklist was used to grade the quality of included studies.

Results: Initial literature search resulted in 6072 papers to be screened. Further screening decreased review to 304 full-text papers for analysis, of which 96 were able to be included. Two-thirds of papers were case reports, and these included 161 total patients with IBD and VTE. Median age was 13.08 years, and approximately three-quarters of patients were female.

Ulcerative colitis was the described type of IBD in about two-thirds of patients. Of the 56 patients with location of disease involvement, nearly all had colonic disease (91%). When considering location of VTE, one-third of affected patients had CVST, 20% had a peripheral VTE, and 8% had pulmonary embolism. Among those with peripheral VTE, 13 patients were described to have a central line associated VTE.

Further investigation into available hematologic parameters demonstrated that an elevated white blood cell count was common (n=45, median count = 14.3 x 109/L, range 1.4-35), as was anemia (n=51, median =8.8 g/dL, range 3.6-13.8), and mild thrombocytosis (n=56, median = 494 x 109/L, range 25-1000). Testing for thrombophilia conditions was rarely available but 65% (n= 23) of subjects tested had elevated Factor VIII activity. Of 57 subjects with follow-up documented, 9 patients died following their VTE event.

Conclusion: In the available literature, we confirmed that colonic involvement is almost always present in pediatric IBD patients who develop a VTE, and CVST is common. IBD patients with VTE tended to have higher white blood cell and platelet counts, and lower hemoglobin levels. Factor VIII activity was increased in IBD patients with a VTE. Mortality was a serious concern among published cases. Our study is limited by the small amount of available data, and the lower quality of papers, given that most papers are case reports. However, despite the known increased risk in this population, more information is clearly needed to better understand this risk and prevent VTE. Multi-center studies are suggested to identify the highest risk pediatric IBD patients and standardize prophylaxis and treatment guidelines.

Gastrointestinal commensal bacteria prevent HIV infection by metabolizing tryptophan and regulating the host aryl hydrocarbon receptor.

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Introduction: Breastfeeding is a major route of HIV transmission among infants, accounting for ~50% of the >150,000 annual pediatric HIV infections. Despite repeated daily HIV exposure during breastfeeding, >85% of infants of untreated HIV-infected mothers do not acquire the virus. While maternal antibodies and antiviral components in breast milk play crucial roles in reducing the risk of oral HIV acquisition, the protective roles of the infant gastrointestinal (GI) microbiome on viral infection have not been extensively studied.

Objective: To identify specific GI commensal bacteria that can delay the risk of oral HIV acquisition and study associated anti-HIV mechanisms.

Method: A novel microbe-phenotype triangulation platform was used to identify bacterial taxa associated with decreased HIV susceptibility in an archived cohort of nursery-reared infant rhesus macaques (RM). The relative abundance of Lachnospiraceae in a human cohort of young children was also estimated. The effect of commensals and metabolites on HIV replication was assessed using a TZM-bl-luc reporter cell line.

Results: Bioinformatic analysis of the infant RM fecal 16S rRNA gene sequences identified Lactobacillus gasseri and Lachnospiraceae to be associated with decreased susceptibility to oral viral acquisition. Furthermore, using a human cohort, we demonstrated that perinatally HIV-exposed uninfected (HEU) children (n=31) gut is colonized with increased levels of Lachnospiraceae, compared to children living with HIV (n=70), suggesting that a higher abundance of Lachnospiraceae in the gut of HEU children might be associated with a reduced HIV acquisition risk. Next, we identified 2 bacterial species within the family Lachnospiraceae, Clostridium immunis and Ruminococcus gnavus, with potent HIV-inhibitory properties in vitro. Since tryptophan metabolism has been associated with HIV pathogenesis and disease severity, we generated an isogenic mutant of C. immunis that lacks the gene encoding aromatic aminotransferasae (ArAT), which metabolizes tryptophan into 3-indolelactic acid (3-ILA). Intriguingly, C. immunis ArAT mutant lacked the ability to inhibit HIV, a finding that indicates the critical role of this gene. To demonstrate the generalizability of this finding, we found that Lactobacillus reuteri also inhibits HIV infection in an ArAT-dependent manner. Notably, the tryptophan metabolite 3-ILA, as well as an agonist of its cognate host receptor—aryl hydrocarbon receptor (AhR), were sufficient to inhibit HIV infection.

Conclusion: We demonstrate that tryptophan metabolism by GI commensal bacteria inhibited HIV infection by regulating AhR.

Single Cell analysis Unravels CD4 T Cell Heterogeneity In Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by breakdown of tolerance and high Type I Interferon (IFNs) signature in the blood. CD4+ T cell dysfunction is well documented in SLE. Yet, CD4+ helper T cells (e.g Th10 and Tph) are expanded in SLE patients and might contribute to extrafollicular antibody response. Furthermore, in patients with high disease activity (DA), regulatory CD4+ T cells (Tregs) expressing naïve markers are expanded although their pathogenic function is still not elucidated. Given the heterogeneity of CD4+ T cells in the peripheral blood of the SLE patients, it is important to characterize this compartment at the single cell level. Therefore, we performed single cell RNA sequencing of purified CD4+ T cells from the blood of 16 children with SLE and 11 matched healthy controls for a total of 275,230 cells. Our single cell analysis of the CD4 compartment revealed significant expansion of memory, ISG-high and Treg subpopulations, in contrast to a reduction in naïve CD4+ cells in SLE patients with lupus nephritis. Subcluster analysis of the naïve CD4 compartment revealed six transcriptionally distinct naïve CD4 subclusters, including one with high BCL2 expression that showed significant expansion in SLE patients compared to healthy donors. Subcluster analysis of the memory CD4 compartment revealed 10 transcriptionally distinct memory CD4 subclusters. We found an expansion of CXCR5+PD1+CCR7- T follicular subcluster within the memory CD4 compartment in SLE patients. We also confirmed the expansion of two extrafollicular subclusters with transcriptionally distinct signature (Th10 and TpH) in SLE patients. Subcluster analysis of Tregs revealed three transcriptionally distinct Treg subsets, including a memory subcluster with high expression of HLA-DR, IL10, CTLA4 and CD39, and a naïve-like subcluster with high expression of FCRL3 and PD-1, which were both expanded in SLE patients. Analysis of ISG-high cells showed that this subcluster was composed of naïve, memory and Treg cells, which were expanded in SLE patients. Our study revealed distinct differences in the CD4+ T cell compartment between children with SLE and healthy controls, which might shed insights on new pathogenic CD4+T cell subsets.

The lung pro-thrombotic niche drives cancer-associated thromboembolism via exosomal ITGB2

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Introduction: Thromboembolism (TE) is a common complication in cancer patients, especially in those patients with advancing metastatic disease, and represents the second leading cause of cancer-related deaths. The prevention of TE remains an unmet clinical need due to the lack of predictive biomarkers. Current routine therapies, such as small molecular weight heparin, used routinely as a treatment for thrombosis prevention often fail due to their associated risk of bleeding. Thus, improved targeted therapies are needed to treat those cancer patients at risk for thrombosis. All cells, including cancer cells and cells in the tumor microenvironment, release large numbers of small extracellular vesicles called exosomes which package a selective cargo of proteins, lipids, mRNA, and DNA, and which can serve as diagnostic and prognostic biomarkers to predict cancer and metastatic potential. However, the role of exosomes in thrombosis and cancer-associated TE remains to be investigated.

Objective: Our aim is to dissect the role of exosomes as initiators of cancer-associated TE, in order to develop exosome-based biomarkers for TE risk and targets for TE prevention.

Methods: Exosomes were isolated from tissues of mice with melanoma (B16F10), breast cancer (MMTV-PyMT), lung cancer (Lkb1-/- KrasG12D/WT), and PDAC (KPC), or cell lines. The pro-thrombotic effect of exosomes was studied in vivo (platelet count, D-Dimer, platelet/fibrin staining), and in vitro (LTA and flow cytometry).

Results: We found that exosomes isolated from (pre-)metastatic lung tissues of mice with melanoma, breast, lung, and pancreatic cancer induce extensive pulmonary embolism when injected intravenously in naïve mice. Large fibrin clots containing exosomes were found within the lung vasculature of these mice, mimicking acute pulmonary embolism in humans. Remarkably, exosomes from tumor cell lines, normal lungs, primary tumors, or other metastasis-bearing organs did not induce thrombosis. Interstitial macrophages infiltrating pre-/post-metastatic lungs were the main source of ITGB2+ exosomes. Examination of the mechanisms of ITGB2-induced TE showed that ITGB2 dimerizes with ITGAX on the exosome membrane and that these heterodimers are in an active conformation that allows their interaction with platelet GPIb, thus inducing platelet activation and aggregation. Importantly, blockade of ITGB2 on lung exosomes or systemically in mice prevented exosome-induced platelet aggregation and TE. Finally, we showed that exosomal ITGB2 levels are elevated in the plasma of Stage IV PDAC patients prior to TE events in comparison to patients with no history of TE.

Conclusions: Our results provide the first evidence of the direct interaction of platelet and exosomes from the pro-thrombotic lung niche in different cancer models, with exosomal ITGB2 having a central role in TE initiation. Moreover, we identify exosome-associated ITGB2 as a new target for the prevention and/or treatment of TE, as well as a potential "liquid biopsy" analyte for the early stratification of patients at high risk of TE.

Poster Presentation Abstracts

Polysomnography Findings in Children's Interstitial Lung Disease (chILD)

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Rationale: Children's interstitial lung disease (chILD) is an umbrella term encompassing a heterogeneous group of complex, rare, diffuse lung diseases affecting infants and children. As sleep related breathing disorders can impact health, development, and growth, early diagnosis is important for better prognosis of individuals with chILD. Previous studies have evaluated sleep disordered breathing within individual chILD diagnoses such as Neuroendocrine Cell Hyperplasia of Infancy (NEHI). The aim of this study was to describe findings on overnight polysomnography (PSG) in a cohort of patients with chILD.

Methods: We performed a single center retrospective chart review under an Institutional Review Board approved protocol. We queried the Weill Cornell electronic medical record to identify children 0 – 21 years old who ever received diagnostic ICD-10 codes for chILD from 2012-2022. Retrospective validation protocol was performed independently by two pediatric pulmonologists to confirm patient cohort. Those who underwent PSG were identified and demographic information, chILD diagnosis, symptoms, reason for referral and polysomnography data were recorded and analyzed. Obstructive sleep apnea (OSA) was defined as AHI >1/hr and sleep related hypoxemia as >5min below SpO2 90%.

Results: Fourteen children out of 52 with chILD underwent a full night polysomnography. Of these, four (28%) had chILD associated with rheumatologic disease, 4 (28%) bronchiolitis obliterans, 2 (14%) ABCA3, 2 (14%) chILD of unknown etiology, 1 (7%) NEHI, and 1 (7%) pulmonary interstitial glycogenosis. Of those who were referred for PSG, 4 (29%) patients were referred for hypoxemia, 7 (50%) for snoring, and 3 (21%) for both. Of those who underwent PSG, 8 (57%) children met the diagnosis for OSA and 3 (21%) for sleep related hypoxemia (Table 1). Overall, the prevalence of OSA in the population of children with chILD and tested with PSG was 53%.

Conclusion: A high prevalence of sleep related breathing disorders was found in a cohort of children with chILD. The rate of OSA is higher than in the general population, which previously was thought to be uncommon in children with chILD. Early screening and low threshold for referral for polysomnography are recommended for early diagnosis and treatment. Larger, multicenter studies are necessary to fully understand the prevalence and impact of sleep disordered breathing in patients with chILD.

Frequency of Medication Errors and the Impact of Discharge Education Among Families with Non-English Language Preference

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Background: Medication errors are common following hospital discharge and Spanish speaking caregivers are at increased risk. Health literacy informed “advanced counseling” techniques such as demonstration, teachback and showback have been shown to reduce caregiver dosing errors and improve understanding of medication instructions, though their effect on medication errors after pediatric hospital discharge has not been investigated.

Study Objectives: To determine the frequency of medication-related errors among English- and Spanish-speaking parents after pediatric hospital discharge and identify whether the use of advanced counseling is associated with decreased medication errors after discharge.

Methods: We performed a prospective cohort study of English- and Spanish-speaking caregivers of children < 18 years old prescribed a new oral daily medication after hospital discharge from March-November 2022. Participants were recruited from an academic tertiary children’s hospital and a community-based hospital. Eligible participants were contacted by phone within four days of discharge and surveyed about management of their children’s medication and elements of discharge education, including use of teach-back and showback. Participants also completed a 4-item validated health literacy assessment (BRIEF). Spanish-speaking participants were surveyed by bilingual investigators. Bivariate analysis compared subject/visit-level factors and discharge processes with medication errors.

Results: A total of 118 caregivers were surveyed, 44 with a primary language of Spanish and 74 English. Liquid medications (88%) and antibiotics (75%) were prescribed most commonly. There were no significant demographic differences between language groups apart from caregiver’s race and ethnicity (p=.01,< 0.001) (Table 1). Overall, 31 medication errors were identified, including 15 dosing errors. 20% of Spanish-speaking families made dosing errors compared to 8.0% of English-speaking families (p=0.059). There were no significant differences in errors between groups with respect to medication start date, frequency of administration or duration (Table 2). There were no significant differences in medication errors based on receipt of advanced counseling techniques from either nurse and/or doctor (Table 3).

Conclusion: Dosing errors were more common among Spanish speaking caregivers after hospital discharge. Advanced counseling techniques did not impact medication errors. Future studies should investigate optimal ways of counseling about discharge medications and standardizing discharge education, particularly for families with a primary language other than English.

Resident Led Quality Improvement Project Incorporating a Clinical Psychologist to Improve Screening and Referral Rates for Postpartum Depression in a Pediatric Primary Care Clinic

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Introduction: Postpartum depression (PPD) is the most common obstetric complication affecting both mothers (10-16%) and their infants. At a Medicaid-based clinic, baseline screening rates in 2021 were 66%, despite efforts to increase screening through prior QI initiatives. Integration of a clinical psychologist into primary care clinic (through a program known as “HealthySteps”) improves screening and referral for PPD.

Objective: By June 2023, a resident-led QI team will increase PPD screening during well child visits to 90%. Secondary aim is to increase referral rates to appropriate mental health care to 100%.

Design/Methods: This is an ongoing observational time-series study over 23 months in a primary care clinic affiliated with a tertiary academic medical center. An interdisciplinary QI team including residents, attending physicians, a QI specialist, and psychologist created a key driver diagram (Figure 1). Interventions were derived using tertiary drivers (Figure 1) and tested via 2 PDSA cycles. Process (screening), outcome (mental health referral) and balancing (safe sleep counseling) were collected via EMR review at 2-week, 1-, 2- and 4-month well child visits. PPD screens were translated into 12 languages. Statistical process control charts, run charts and subgroup analyses were used to display and analyze data. Run chart rules and API rules were applied to detect signal of change and special cause variation respectively.

Results: 715 charts were reviewed. Overall PPD screening rate improved from 66 to 85% (Figure 2), while previously improved scoring remained high at 98%. Positive screening rate remained unchanged at 10%, while referral rate of patients who scored positive improved from 60 to 87% (Figure 3). Subgroup analysis showed the lowest screening rate at the 2- week visit. Only 50% of newly introduced, non-English PPD screens were completed (23/42). There was no change in safe sleep counseling (balancing measure).

Conclusions: Integration of a clinical psychologist into the resident clinic was effective in improving both screening and referral rates. The lower screening rates observed at 2-week visits may be due to the focus on weight trends. Given the lower number of non-English screens completed, improving workflow, and educating staff on how to perform non-English screens is important for further success and reducing disparities. Future interventions for this ongoing project include implementing note templates for 2-week visits, modifying existing note templates to track outcomes and languages of completed screens.

Evaluating anti-tumor activity and determinants of response to trastuzumab deruxtecan, an anti-HER2 antibody-drug conjugate, in pediatric solid tumors

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Introduction: Antibody-drug conjugates (ADC) represent a novel strategy for the directed delivery of a toxic payload to cancer cells. Trastuzumab deruxtecan (T-DXd) is a HER2-ADC containing a topoisomerase inhibitor payload approved for the treatment of HER2-positive breast and gastric cancer. Clinical trials of T-DXd have demonstrated significant clinical responses even in patients with low HER2-expressing tumors. HER2 expression is observed across multiple pediatric cancers including osteosarcoma (OS), Wilms tumor (WT), and malignant rhabdoid tumors (MRT). Given the clinical activity observed with HER2-targeted therapy in breast and gastric cancers, T-DXd is being explored in an early phase clinical trial through the Children's Oncology Group in patients with HER2+ osteosarcoma (NCT04616560). However, inconsistent detection of HER2 in tumor samples has challenged the role of HER2 expression as a response biomarker and warrants further investigation in pediatric cancers.

Objectives: This study evaluates the in vitro and in vivo anti-tumor activity of T-DXd in OS, WT and MRT and correlates response with HER2 expression by multiple assay approaches. We explore the contribution of the T-DXd payload in predicting activity in these pediatric solid tumor models. Additionally, we utilize a novel systems biology approach (metaVIPER) to identify and evaluate putative non-genetically encoded biomarkers of response to T-DXd.

Methods: In vitro sensitivity of pediatric cancer cell lines to T-DXd and the toxic payload deruxtecan was performed. In vivo activity of T-DXd was evaluated in OS, WT, and MRT patient-derived xenograft (PDX) models at multiple dose levels. HER2 expression in PDX tumors was evaluated by immunohistochemistry (IHC), mass spectrometry, flow cytometry, immunoblot, and quantitative PCR. IHC was performed using five different validated anti-HER2 antibodies. In vitro and in vivo responses were correlated with HER2 expression by IHC. MetaVIPER analysis was performed to computationally infer protein activity from whole transcriptomic data.

Results: WT and MRT cells exhibited significant sensitivity (IC50: 0.2 – 20 nM) to the topoisomerase payload (deruxtecan) of T-DXd and variable sensitivity to T-DXd itself (IC50: 52 – 400 nM). T-DXd demonstrated significant anti-tumor effect in OS, WT and MRT PDX models. Treatment of OS PDX models resulted in an overall disease control rate of ~67% with partial response seen in ~22% (4/18) and stable disease in 44% (8/18) of treated animals. Similar to OS models, low to no HER2 expression is observed across WT and MRT models. No correlation was observed between in vivo response and HER2 expression. MetaVIPER analysis identified topoisomerase (TOP1, TOP2A) as having high aberrant inferred activity in OS, WT and MRT. Conversely, HER2 was not identified as aberrantly activated in these tumors.

Conclusion: T-DXd shows promising preclinical activity across multiple pediatric solid tumors supporting further clinical exploration of T-DXd. We observe inconsistent and low to no expression of HER2 across tumor types and lack of correlation with anti-tumor response suggests re-evaluation of HER2 status as a biomarker of response. Hence, development of alternative strategies (metaVIPER) for identifying putative response biomarkers will be critical to the development of tumor agnostic studies.

Exploring novel immunologic or microbiologic mechanisms leading to iron deficiency in quiescent pediatric Crohn's disease

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Introduction: Iron deficiency anemia (IDA) is the most common extra-intestinal complication seen in patients living with inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis. IDA affects up to 70% of patients admitted for treatment and 20% of patients seen in the outpatient setting. IDA is more prevalent in patients with Crohn's disease than ulcerative colitis. It is known that iron deficiency anemia may result in neurodevelopmental delay, cognitive impairment, attention deficit hyperactivity disorder (ADHD), and fatigue amongst side effects. Given the significant impact on quality of life, anemia must be identified and corrected quickly.

Oral and intravenous iron replacement therapies uniquely alter the gut microbiota in patients with IBD. The presence of iron in the diseased gut increases IBD-disease activity, as measured by fecal calprotectin and histologic evaluation of tissue samples, by inducing oxidative stress at the site of bowel inflammation, which further exacerbates iron malabsorption. As such, management of IDA in patients with active disease often requires iron infusions. However, as bacterial translocation is a risk in IBD, there is concern that intravenously administered iron may be utilized by translocated bacteria as an energy source, leading to further inflammation and sepsis. Prior studies have shown the efficacy of oral iron is limited in IBD. Interestingly, however, studies of iron absorption capacity in patients with quiescent disease reveal that patients do have the ability to absorb iron; yet it is known that 1 in 5 patients in disease-free remission may still have some measure of iron deficiency anemia on laboratory examination. Therefore, there is an urgent need to understand the etiology of iron deficiency anemia in quiescent pediatric IBD to optimize treatment.

There is a lack of research evaluating the microbial, inflammatory, and immunologic factors implicated in iron malabsorption and metabolism in patients with quiescent Crohn's disease. Given decades of research linking the pathogenesis of IBD to disruptions in the microbiome, it is hypothesized there are differences in the microbiota, inflammatory cascade, and immunologic response in patients with quiescent Crohn's disease that explain the pathogenesis of IDA. Evaluation of these differences will allow for the development of a predictive model used in the treatment of IDA in pediatric Crohn's disease.

Objectives: We evaluated the impact of the microbiome and immune status on iron regulation in pediatric patients with quiescent Crohn's disease, specifically seeking to understand if altered compositions in intestinal microbiota or immune phenotypes may explain the etiology of, or a mechanism for, iron deficiency anemia in this patient population.

Methods: We utilized both cross-sectional, descriptive, and analytical study designs to evaluate stool samples stored in the JRI Live Cell Bank (LCB) as well as samples collected from study participants during study enrollment. The JRI LCB contains more than 728 unique sample sets from individuals living with IBD.

Results: Of the 728 sample sets within the JRI LCB, 363 samples were excluded. 365 met the initial inclusion criteria. Through retrospective chart review, study participant characteristics were collected including, age, sex, weight, diet, seasonality, and iron supplementation type and dose, and medication use which have all been shown to impact the microbiome. Additional information, including disease location, PCDAI scores, blood iron level and saturation, and stool characteristics (including fecal calprotectin) will also be documented. Of the 365 sample sets, 222 sets were further excluded if they lacked stool samples for evaluation or if histopathologic evaluation indicated active disease in the duodenum or terminal ileum.

143 stored samples in the JRI LCB were included for analysis from 56 male (39.2%) and 87 female (60.8%) study participants, aged three to twenty-one years, with biopsy confirmed, quiescent Crohn's disease. Samples were included if the study participant also had concomitant IDA with at least two separate lab evaluation sets, including hemoglobin, MCV, iron, and/or ferritin values below age- and sex-defined cutoff values. Quiescent disease was determined by histopathologic examination as well as normalization of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and Pediatric Crohn's Disease Activity Index (PCDAI). Comparator groups included similarly matched quiescent Crohn's disease study participants without iron deficiency anemia as well as healthy pediatric study participants with and without IDA, all of whom were represented in the JRI LCB. Samples from participants treated with antibiotics or probiotics within 6-weeks of sampling were excluded from this study.

We applied Next Generation Sequencing (NGS) to stool samples to evaluate 16S microbial rRNA. Samples were analyzed for taxonomic composition, alpha diversity (Chao 1 index, Shannon-Wiener index, and Simpson index), and beta diversity (Bray-Curtis dissimilarity and UniFrac distance). Results obtained will be compared against reference datasets for both normal, healthy gut microbiome as well as that associated with iron deficiency anemia without concomitant IBD. These data will be utilized to develop a predictive model for the treatment of iron deficiency anemia in quiescent pediatric Crohn's disease.

Conclusions: To our knowledge, this is the first study to utilize microbiome analysis in quiescent pediatric Crohn's disease with concomitant IDA for the development of treatment protocols for the management of IDA in quiescent pediatric Crohn's disease.

Extracellular vesicles and particles (EVPs) participate in exercise-induced communication and metabolic homeostasis

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Physical activity is associated with positive changes in cardiovascular, respiratory, metabolic and hormonal function. Recent evidence reveals that chronic exercise promotes physiological adaptations that stimulate the reprogramming of the systemic milieu, by altering the recruitment, mobilization, and function of a myriad of cell types and molecules. Tissue crosstalk is an underappreciated mechanism responsible for the physiological effects of exercise. While exercise research has focused on the role of muscle-derived secreted proteins (e.g., myokines) as mediators of exercise-induced muscle regeneration and metabolic homeostasis, other organs and mechanisms of systemic communication might drive the physiological effects of exercise.

Extracellular vesicles and particles (EVPs) are small, secreted vesicles/nanoparticles and are critical in intercellular communication at local and distant sites. Normal and tumor cells produce EVPs, and emerging evidence suggests a role for EVPs in tissue homeostasis and tumor protection, Thus, we hypothesize that EVPs mediate tissue crosstalk during exercise and are responsible for the benefits of exercise in breast cancer prevention and survival.

Female 8 week-old BalbC, C57BL/6 and FVB/NJ mice were exposed to chronic exercise training or sham control for 4 weeks. Exercise was performed on a stationary treadmill at 22m/min, 0% grade for 60 mins/session. EVPs were isolated from organ-explant using ultracentrifugation. In this mouse model of exercise, we revealed that organ-specific EVP secretion and biodistribution (organ-targeting) is exercise-dependent. Using mass spectrometry, we identified exercise-specific EVP proteins (exerkines) that may serve as biomarkers of organ-specific response to exercise in this mouse model. We treated mice with sedentary or exercise organ-derived EVPs (education) to mimic the EVP release in the plasma during exercise. We obtained exciting results showing that exercise-induced EVPs positively impact metabolic liver function.

We obtained blood samples from two recently completed randomized trials of highly controlled exercise training. Patients at a high risk for developing breast cancer (e.g., family history and/or BRCA1/2 mutation carrier) and post-menopausal breast cancer patients were randomized to one or two exercise doses (EX) or control (Sed) for 6 month (n=75) or 4 months (n=174), respectively. By comparing the contents of murine EVP exerkines to those from participants and patients, we hope to identify EVP-exerkines signatures in both human and mice that are responsible for the beneficial effects of exercise.

Self-assembling peptide nanofiber HIV vaccine elicits robust vaccine-induced antibody functions and modulates Fc glycosylation

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Background: Nanomaterials represent a novel class of vaccine platforms that present potential advantages for HIV vaccine design. In previous work, we have demonstrated that nanofibers formed from the β-sheet self-assembling peptide named Q11 are a useful vaccine platform for tailoring adaptive immune responses. Q11 nanofibers have self-adjuvanting properties and also allow for controlled valency of conjugated peptides or proteins over several orders of magnitude. In a recent study, we demonstrated that conjugating the HIV gp120 antigen to Q11 nanofibers induced antibody responses with stronger binding to heterologous HIV Env antigens than soluble gp120 in immunized mice, and that enhancement of the antibody response was driven by the multivalent presentation of gp120. Yet, it is still unclear how Q11 nanofiber impacts the functions and the glycosylation profile of the induced antibodies.

Method: We conjugated HIV gp120 to Q11 nanofiber via a site-specific sortase-mediated reaction. Rabbits were immunized with Q11-conjugated gp120 (gp120-Q11) or soluble gp120 (n = 8 in each group). Neutralization against HIV was measured using a standardized TZM-bl cell neutralization assay. Fc-mediated functions antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) were measured by flow-based assays. To phenotype the glycosylation of gp120-specific antibodies, rabbit serum was incubated with gp120-conjugated magnetic beads, followed by glycan analysis using a capillary electrophoresis method. For total serum IgG glycosylation, protein A was used to purify total serum IgG followed by the same glycan analysis procedure.

Results: Consistent with our previous study, conjugation of gp120 to Q11 increased the binding breadth of the induced antibodies in rabbits. Moreover, immunization of gp120-Q11 also induced more robust antibody binding to HIV-infected cells than soluble gp120 ($P = 0.0166$). Functionally, gp120-Q11 induced higher neutralization of easy-to-neutralize (tier 1) autologous viruses ($P = 0.0406$) and earlier development of ADCP ($P = 0.013$). Moreover, gp120-Q11 elicited a superior ADCC response with higher ADCC-inducing antibody titers ($P < 0.001$) and higher magnitude ($P = 0.001$) of ADCC against HIV-infected cells. Glycosylation of gp120-specific IgG induced by gp120-Q11 immunization was distinct from soluble gp120, yet such distinction was not observed in total IgG. In rabbits immunized gp120-Q11, we found higher levels of fucosylation ($P = 0.0047$) and mono-galactosylation ($P = 0.038$) in the gp120-specific IgG. Such change in glycosylation of gp120-specific was correlated with the increase of ADCC.

Conclusions: In this study, we demonstrated that Q11 not only can increase the antibody binding response, it also can enhance the antibody functions such as neutralization, ADCP, and ADCC. Moreover, presenting gp120 on Q11 nanofiber appeared to modulate the glycosylation profile of antigen-specific IgG in immunized animals, and such change was correlated with increased ADCC. Taken together, Q11 nanofiber presents a novel vaccine platform to tailor the adaptive immune response for HIV and other infectious diseases.

Retrieval Practice with Exam-Style Review Questions Improves Individual Residents' Performance on Standardized Examinations

Presenting Author: Adin Nelson, MD MHPE (Faculty, Adin Nelson, MD MHPE)

Authors Nelson AN, Mohammed A, An A, Traba CM

Introduction: Residents have limited time and much to learn in order to become competent clinicians and pass high-stakes licensing and board-certification examinations. Retrieval practice - active studying using exam-style review questions - has been shown to be a powerful educational tool for groups of learners in aggregate, but no studies have tested the effects of retrieval practice for individual learners in Graduate Medical Education (GME).

Objectives: In this study, we examined how individual trainees' retrieval practice affected their learning during residency.

Methods: We conducted a retrospective observational study of learners' self-directed use of retrieval practice in one pediatrics residency program. We recorded the number of unique exam-style multiple-choice practice questions each resident independently answered each year, and we compared that to their scores on the annual American Board of Pediatrics In-Training Exam (ITE) using mixed-effects linear models. We also included residents' prior exam scores as individual baselines, which has not been reported in any previous studies.

Results: We found a complex set of relationships between ITE scores and practice questions. Residents' ITE scores at the beginning of an academic year significantly but negatively predicted how many practice questions they would answer that year for both PGY-1 ($p = 0.023$) and PGY-2 ($p = 0.020$). The number of questions residents answered then significantly and positively predicted the change in their ITE score from PGY-1 to PGY-2 ($p = 0.026$) and from PGY-2 to PGY-3 (p = 0,025). Residents' prior ITE scores also independently and significantly predicted their subsequent ITE scores in PGY-2 ($p = 0.024$) and PGY-3 ($p = 0.007$), and that effect of their baseline scores was larger than the effect of the number of practice questions they answered.

Conclusions: Our results show that while the baseline effect of previous exam scores predicting future exam scores is powerful, retrieval practice using exam-style review questions also has a significant impact on individual residents' learning. These findings can help GME program leaders make evidence-based decisions in selection, academic coaching, and education for pediatrics residents. Further research should validate these effects in broader settings and prospectively test different ways of using retrieval practice in GME.

Targeting latency switch for the treatment of EBV+ lymphoma

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Epstein-Barr virus (EBV) infection contributes to the development of a significant subset of human lymphomas. EBV-infected lymphoma cells exist in three latent states: latency I, II or III. Latency II and III infected cells express immunogenic viral proteins such as EBV latent membrane protein 1 or 2 (LMP1/2) and EBV nuclear antigen 3 (EBNA3) that can be recognized by cytotoxic T cells (CTLs). As such, EBV-specific T cell immunotherapy is being developed for the treatment of latency II and III EBV+ lymphoma. However, these proteins are not expressed in latency I EBV+ lymphoma, rendering these tumors resistant to EBV-specific T cell therapies. To address this, we investigated the use of pharmacologic agents to convert latency I Burkitt lymphoma (BL) tumors to the latency II/III program, rendering these cells to be susceptible to T-cell therapies. We found that hypomethylating agent decitabine induces the latency II/III program in BL tumors and renders tumors susceptible to killing by EBV-specific CTLs. We revealed that decitabine-induced latency conversion is regulated through the inhibition of DNMT1 and validated this by showing that DNMT1 inhibitor, GSK032 potently induces latency I to latency II/III conversion of BL cells.

Despite our promising findings of tumor growth inhibition in response to decitabine-induced latency conversion followed by EBV-CTL therapy, there are key gaps in knowledge that will be essential to the ultimate clinical translation of this approach: 1) little is known about the immune cells required for the clearance of latency-switched tumors and if allogeneic T-cells are needed or if the intact immune response is sufficient; 2) how to overcome the resistance of the EBV+ latency-I lymphomas that do not undergo latency conversion in response to decitabine. Understanding mechanisms of resistance will allow for the development of approaches that maximize conversion to latency II/III.

To study the human immune response to latency-converted EBV+ lymphomas, we developed a humanized mouse model of BL. SGM3-NSG mice (NOD.Cg-PrkdcscidIl2rgtm1WjlTg (CMV-IL3, CSF2, KITLG)) are transplanted with human cord blood CD34+ cells and then engrafted with an HLA-matched latency I EBV+ BL tumor, prior to treatment with decitabine. We found that decitabine treatment of humanized mice with BL increases T cell infiltration to the tumor, suggesting a robust T-cell response in the absence of allogeneic EBV specific CTLs. We performed RNA-sequencing to identify the genes that contribute to the resistance to decitabine-induced latency switch. To examine the epigenetic machinery that may contribute to the mechanism of decitabine-induced latency switch, we performed ChIP-qPCR and bisulfite-sequencing.

In summary, our work has identified DNMT1 as a key regulator of latency restriction and revealed the potential use of DNMT1 inhibitors to sensitize latency I EBV+ lymphomas to T-cell mediated tumor clearance. Future studies using our newly developed latency-I BL humanized mouse model will help identify the key immune effectors that respond to latency converted cells. This along our findings on the resistance mechanisms will guide the development of rational combination therapies to improve the treatment of otherwise immune refractory EBV+ latency-I lymphomas.

HIV triple broadly neutralizing antibody (bNAb)-based passive immunization delayed viral rebound in orally SHIV-infected infant rhesus macaques

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Background: Antiretroviral therapy (ART) has significantly reduced the morbidity and mortality associated with HIV-1 infection by suppressing viral replication, however due to the rapid establishment of long-lived cells carrying replication-competent proviruses, ART-alone will not cure HIV. Upon the discontinuation of ART viral rebound occurs in most individuals within weeks. Passive immunization with broadly neutralizing antibodies (bnAbs) has been associated with a delay in time to rebound post ART-interruption, but the emergence of resistant HIV-1 variants limits the potential of this strategy when used as monotherapy. We have recently tested the neutralization and non-neutralizing function of a combination of bnAbs and identified a triple bnAb combination with high neutralization potency and breadth as well as robust non-neutralizing effector functions.

Objective: Using an established oral infant rhesus macaque (RMs) SHIV-infection model, our objective was to determine the impact of a triple-bnAb infusion on HIV viral rebound kinetics following ART interruption.

Methods: Infant rhesus macaques were orally infected with SHIV.C.CH505 at 4 weeks of age (n=10). A triple-bNAb combination of simianized 3BNC117, PGDM1400, and PGT151 (each at 40mg/kg) was subcutaneously administered during ART initiation (8 weeks post-infection) and before ART interruption (49 weeks post-infection). Viral load kinetics was monitored for 12 weeks post analytical treatment interruption (ATI). ELISA was utilized to determine the plasma concentration of each bnAb throughout the study. Env-specific IgG responses and anti-drug antibodies (ADA) against each bNAb were also monitored over time.

Results: Env-specific (gp120 and gp41) antibody levels peaked 8 weeks post-infusion, slightly decreased during ART and increased following virus rebound. The bnAb concentration in plasma peaked 1-2 weeks following the initial infusion. All animals had undetectable levels of 3BNC117 8 weeks after infusion and only 2/10 animals had detectable levels of PGDM1400. The levels on PGT151 are still under evaluation. ART was interrupted at 49 weeks post-infection and the virus rebounded in all animals. The average time to virus rebound was 7 weeks (range 3-10 weeks). The time to rebound was significantly longer than in a previous group of animals who did not receive an immune-base intervention (~2.5 weeks). By the time of virus rebound, most animals had undetectable levels of 3BNC117 or PGDM1400, but in a few instances, virus rebound occurred while passively antibodies were still present, suggesting the possible emergence of resistant variants. One animal only showed a transient increase in 3BNC117 and PGDM1400 following the second infusion. The antibodies were no longer detectable 2 weeks after infusion and this animal experienced the shortest time to virus rebound.

Conclusion: Passive immunization with a triple-bnAb infusion, in SHIV-infected ART-treated infant rhesus macaques, prolonged the time to viral rebound. Interestingly, in some cases viral rebound occurred while bnAbs were still detectable. Future studies will assess the presence of bnAb resistant mutations in the virus reservoir and evaluate the sensitivity of the rebound virus to bnAbs.

Improving the neonatal murine model of human rotavirus-induced disease using antibiotic-mediated intestinal microbiota depletion

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Introduction: Rotavirus causes approximately 200,000 deaths/year in children under 5 years of age and disproportionately affects low-to-middle-income countries (LMIC), despite availability of multiple licensed vaccines for children as young as 6 weeks of age. This is primarily caused by lower vaccine efficacy in LMICs which has been attributed to malnutrition, gastrointestinal tract health, maternal antibody interference, and high enteric disease burden. Rotavirus is highly transmissible and is spread by the fecal-oral route, aerosolized droplets, contaminated food and water, and fomites. Additionally, transplacental transfer of IgG and breast milk-transferred secretory IgA interfere with vaccine responses in infants and children. An animal model is necessary to study rotavirus disease progression and factors that impact vaccine efficacy; however, replication of human derived rotaviral strains is restricted in the small intestine in mouse models of rotavirus. We hypothesize that the presence of commensal bacteria in the murine gut may be protective against human rotavirus infection.

In a previous study, 15/17 human rotavirus-infected 5-day old SV129 mice pups had detectable diarrhea by palpation of the abdomen and observation of liquid stool from the anus at day 1 post-challenge. The median human rotavirus antigen level in intestine was 2000U/g (range 500U/g – 7000U/g). There was no discernable small intestinal pathology due to rotavirus infection compared to that of uninfected mouse pups. We hypothesize that antibiotic therapy will enhance human rotavirus replication due to the subsequent decrease of protective commensal gut bacteria.

Objective: We will optimize a human rotavirus challenge mouse model using antibiotic treatment to deplete murine gut microbiota for increased viral replication in the suckling pup model.

Methods: We completed microbial depletion optimization and elicited anti-VP6 seroconversion in an adult mouse model. We tested three routes of pup antibiotic exposure: intrauterine, breast milk, and direct oral administration. Antibiotic-exposed (n=18) and unexposed pups (n=19) were challenged with 106 FFU of Wa strain human rotavirus and tested for presence of rotavirus antigen in intestinal content via ELISA and RT-qPCR. To determine the impact of rotaviral infection pups were monitored for clinical signs such as lethargy and diarrhea and development of intestinal lesions at 1-4 days post-challenge.

Results: Adult mice were treated with antibiotics in drinking water and gut microbiome depletion was confirmed by bacterial 16S sequencing of fecal pellets. Antibiotic-treated adult mice were infected with 106 FFU Wa rotavirus and showed high IgG binding to capsid protein VP6 in serum after a second inoculation. After primary infection, mice had significant weight loss for 8 days post-infection.
After viral challenge, pups born to dams that were treated with antibiotics prior to giving birth (pre-treatment) had a median human rotavirus antigen level in intestine of 350U/ml (range 343U/ml – 400U/ml) while pups exposed to antibiotics either directly (oral) or through dam drinking water (breast milk exposure) fell below the positive assay cut off (150U/ml). Only 2/18 antibiotic treated pups developed observable liquid diarrhea. In the pre-treatment group, 2/2 pups exhibited mild cytoplasmic vacuolation and swelling of enterocytes in the small intestine. In the other antibiotic treated groups, the small intestines of 4/4 pups were normal or had mild surface epithelial vacuolation compared to controls mock-infected with PBS (n = 10). We analyzed small intestine of antibiotic pre-treated pups by RT-qPCR and observed a median of 448 viral copy number (NSP3 equivalent), whereas antibiotic breast milk exposed pups had decreased viral copy number (range of 0 – 19 viral copy numbers NSP3 equivalent).

Conclusions: Studies are ongoing to test a higher titer inoculum (>107 FFU) in the pup infection model to compare antibiotic treated and untreated, rotavirus infected pups. Our data suggests that antibiotic treatment does not enhance human rotavirus infection in a mouse model and further studies with immunocompromised models (STAT1 knockouts) are planned.

High levels of HCMV glycoprotein-specific antibodies predict congenital HCMV acquisition

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Introduction: Congenital cytomegalovirus (cCMV) is the most common infectious cause of birth defects worldwide, affecting 0.5-2% of live births. The risk of cCMV infection is higher after maternal primary infection compared to re-infection or reactivation of latent virus during pregnancy, suggesting there is a partially protective role of adaptive maternal immunity against transmission. Pregnant people living with HIV/HCMV have an elevated risk (2-7%) of vertical CMV transmission. This increased risk in the setting of untreated HIV infection may be due to HIV-associated factors such as impaired CD4+ T cells, but immune correlates of transmission in HIV-infected pregnancies, and whether they are distinct to those without HIV, remain unclear.

Objectives: We aim to define the humoral immune correlates of protection against cCMV in the high-incidence setting of maternal HIV/HCMV co-infection, which will help address the gap in knowledge of protective immunologic responses against cCMV, a gap that is currently impeding HCMV vaccine development for this important demographic in the global HCMV epidemic. Specifically, we hypothesize that the magnitude, specificity, function, and placental transfer of maternal antibodies are associated with protection against cCMV transmission in HIV and CMV-seropositive pregnant people. Methods: Using samples from 3 historical cohorts, we performed PCR on HIV/CMV-coinfected maternal plasma drawn near delivery and infant plasma collected at <2 weeks of age. We identified 15 cases of cCMV transmission. Cases were matched 1:2 by propensity score to HIV/CMV coinfected non-transmitting dyads (n=30) based on maternal age, gravida, parity, and CD4 counts. We measured total and virus-specific antibody magnitude by ELISA and Binding Antibody Multiplex Assay (BAMA). We assessed functional antibody responses by Antibody-Dependent Cellular Phagocytosis (ADCP), Antibody-Dependent Cellular Cytotoxicity (ADCC), Fc gamma receptor binding, and neutralization assays. Mann-Whitney U tests were applied to assess differences in total and functional HCMV-specific antibody responses, using p-values of <0.05 as the significance threshold for our hypothesis-generating study.

Results/Conclusions: We observed a trend of increased HCMV-specific antibody responses, including higher virion glycoprotein specific IgG concentrations and neutralizing antibody titers, in transmitting compared to non-transmitting maternal plasma. These results suggest that a high quantity of HCMV-specific antibodies is not independently protective against cCMV acquisition. In previous literature, increased ADCP and ADCC responses have been established as protective against cCMV acquisition in non-HIV infected cohorts. In this cohort, we observed similar levels of ADCC and ADCP responses in transmitting and non-transmitting groups, underscoring the variation in immune correlates in distinct maternal demographic groups and HCMV exposure status.

These results suggest that high HCMV glycoprotein-specific IgG binding and neutralizing titers could be used as a predictive marker of the risk of cCMV infection in the setting of maternal HIV/HCMV co-infection. Ongoing work will determine if the observed higher antibody responses in transmitting mothers are associated with HCMV viremia to determine whether decreasing maternal HCMV viral loads via vaccination can be a protective intervention against cCMV transmission.

Workplace-Based Clinical Assessments of Medical Students Reflect Assessors and Random Chance More Than Students

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Please note: I (Rachel Arnesen) am the first author but I will be graduating and out of the country for this event. Dr. Adin Nelson has volunteered to present this project in my absence.

Background: Medical students' clinical performance is assessed both qualitatively (with narrative comments) and quantitatively (with numerical scores). Those scores play a large role in students' final grades and residency applications, but previous studies have questioned whether clerkship clinical assessments truly reflect the student or the assessor.

Objective: 1) To explore sources of variability in quantitative student assessments including assessor characteristics, student demographics, and rotation site and sequence; 2) To test assessment reliability and determine the number of assessments required for a valid metric of student performance.

Design/Methods: We conducted a retrospective cross-sectional study of written assessments (SPEs) in the Pediatrics Clerkship at Weill Cornell from 2018 through 2021. The SPE is a standardized assessment form of twelve questions with behavior anchors on a 4-point scale. We used a linear mixed-effects model with student and evaluator characteristics as random effects to test whether the SPE scores correlated more with students or assessors. We then used a multivariable mixed-effects model to investigate the effects of various demographic and contextual factors. We also conducted a reliability analysis using the generalizability coefficient.

Results: We analyzed 2,958 SPEs submitted by 380 assessors (67% female; 36% attendings, 4% fellows, 60% residents) for 446 students (50% female). The median number of SPEs per student was 6. Interns and residents gave significantly higher SPE scores than attendings (p <0.001), but there was no impact by gender, rotation site, rotation length, or time of year. We found a greater variance between assessors (0.090 points, SD 0.300) than between students (0.013 points, SD 0.115) and a large residual (0.080 points, SD 0.282), suggesting that assessor characteristics and random effects played a larger role in students' scores than student characteristics. In order to reach 80% scoring reliability using the current assessment system, we would need a minimum of 24 SPEs per student.

Conclusions: We found that assessors' characteristics and random chance contributed more variability to quantitative assessments of students' clinical performance than student characteristics. Additionally, achieving 80% reliability with our current assessment system would require 4 times more assessments than students currently receive. Designing more-nuanced assessments with broader scales, improving training and guidelines for assessors, and soliciting more assessments per student may increase the validity of quantitative clerkship student clinical assessment.

Pharmacokinetics and antiviral potency of an HIV-specific triple broadly neutralizing antibody (bNAb)-based passive immunization in infant rhesus macaques.

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Background: Despite the efficacy of anti-retroviral therapy (ART) in the suppression of productive HIV replication, its inability to cure HIV and associated clinical toxicities warrant the need for adjunctive prophylactic and therapeutic strategies to achieve safe long-term HIV control. Broadly neutralizing antibodies(bNAbs) present a promising opportunity for HIV prevention and cure, given their high breadth and potency against cross-clade HIV strains. However, eliciting these bNAbs naturally with active immunization has been a great challenge due to lack of effective immunogens. A potential strategy to bypass the challenges of active immunization is passive immunization of these potent bNAbs. Furthermore, combining anti-HIV bNAbs with non-overlapping epitope specificities could offer increased breadth and potency of cross-clade anti-HIV function which allows for more effective HIV control, with minimal viral escape. While there are several studies evaluating the pharmacokinetics and efficacy of passive bNAb combination in adults, very little data is available for infants, a population that experiences more than 150,000 new HIV infections annually.

Objective: Evaluate the pharmacokinetics and anti-HIV functions of a passively administered triple-bNAb combination in uninfected infant rhesus macaques.
Method: A triple-bNAb combination of simianized 3BNC117, PGDM1400, and PGT151 (each at 20mg/kg) was subcutaneously administered to three-month-old infant rhesus macaques (RMs; n=3) in a single dose. The plasma concentration of bNAbs, development of antidrug antibodies (ADA), and total HIV-specific IgG were measured by ELISA. Plasma antibody neutralization, antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) against a clade C tier-2 SHIV CH505 was assessed in vitro.

Results: The single-dose triple bNAb combination was safe in infant RM's. The concentration for the three bNAbs individually peaked within 1-3 days and gradually declined over the course of 12 weeks, with a variable median half-life (t1/2) for 3BNC117 (6.51 days), PGDM1400 (5.47 days) and PGT151 (7 days). All three animals attained SHIV-specific neutralizing antibody titer (median peak ID50 =109350) and had detectable HIV-specific ADCC (median peak titer =2712.7) and ADCP (median peak ADCP score=24.3) response in vitro. ADA was detected in 2/3 animals, with one developing endogenous antibody response against all three bNAbs, while the other developed ADA for only 3BNC117. Importantly, there was an observable association between higher ADA and a faster decline in plasma antibody concentration.

Conclusion: The single-dose triple combination of 3BNC117, PGDM1400, and PGT151 showed promising potential for effective and long-acting anti-HIV prophylactic or therapeutic strategy in infants. The therapeutic advantage of this triple bNAb combination in reducing viral reservoir size and time to rebound is currently being investigated in SHIV-infected infant rhesus macaques by our team.

Maternal Broadly Neutralizing Antibody Activity and Perinatal Transmission of HIV-1

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Introduction: Over 150,000 children are infected with HIV-1 every year. Despite increased availability to antiretroviral therapy (ART), up to 5% of women with HIV still transmit the virus to their infants. While development of broadly neutralizing antibodies (bNAbs) through vaccination and therapeutic interventions are intended to prevent vertical transmission of HIV-1, recent evidence suggests that there may be limitations to bNAb-based approaches. Our group has demonstrated viral escape of variants in the presence of maternal plasma bNAbs targeting the V3 glycan site and a dominant bnAb specificity in postnatal transmitting women plasma.

Objectives: We hypothesize that people living with HIV with neutralizing antibody breadth and bnAbs targeting a single epitope may be at high risk of viral escape leading to vertical transmission.

Methods: We acquired plasma from 15 perinatal transmitters and 47 non-transmitters with HIV from the US-based, pre-ART era Mother-Infant Cohort Study (MICS). Plasma was collected at delivery and assessed for neutralization activity against a global HIV-1 panel. Additionally, we previously screened postnatal HIV-transmitters from the Breastfeeding, Antiretroviral, and Nutrition Study (BAN) and Center for HIV/AIDS Vaccine Immunology 009 (CHAVI009) for plasma neutralizing activity (BAN: n = 21 and CHAVI009: n = 3 postnatal HIV-transmitting women). MICS and CHAVI samples were also screened against murine leukemia virus (MLV) and BAN samples against Vesicular Stomatitis Virus-G (VSV-G) for non-specific neutralization.

Results: Seven out of 15 (47%) perinatal transmitters from the MICS cohort neutralized over 50% of viruses of a heterologous, 10-virus global panel after correcting for non-specific neutralization activity (MLV). While this rate is higher than that reported in HIV-infected adults (20-30%), high neutralization breadth was also found among postnatal transmitting women with HIV in the BAN and CHAVI cohorts (18 of 24, 75%) indicating that transmission during perinatal and postnatal settings may involve a similar high rate of maternal bnAb responses that could lead to viral escape. Transmitters also had higher neutralization breadth than non-transmitters in the MICS cohort.

Conclusions: The finding of high plasma bnAb rates in perinatal HIV-transmitted, ART-untreated women is similar to that observed for postnatal HIV-transmitting women and might indicate role for viral escape of neutralization in perinatal transmission. Immune interventions involving multispecific bNAbs that are synergistic with ART may be key for bnAb-based strategies for ending the pediatric HIV epidemic.

Human Cytomegalovirus Glycoprotein B vaccine update

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Gut microbiota-derived metabolites confer protection against SARS-CoV-2 infection

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The gut microbiome is intricately coupled with immune regulation and metabolism, but its role in Coronavirus Disease 2019 (COVID-19) is not fully understood. Severe and fatal COVID-19 is characterized by poor anti-viral immunity and hypercoagulation, particularly in males. Here, we define multiple pathways by which the gut microbiome protects mammalian hosts from SARS-CoV-2 intranasal infection, both locally and systemically, via production of short-chain fatty acids (SCFAs). SCFAs reduced viral burdens in the airways and intestines by downregulating the SARS-CoV-2 entry receptor, angiotensin-converting enzyme 2 (ACE2), and enhancing adaptive immunity via GPR41 and 43 in male animals. We further identify a novel role for the gut microbiome in regulating systemic coagulation response by limiting megakaryocyte proliferation and platelet turnover via the Sh2b3-Mpl axis. Taken together, our findings have unraveled novel functions of SCFAs and fiber-fermenting gut bacteria to dampen viral entry and hypercoagulation and promote adaptive antiviral immunity.

SARS-CoV-2 mRNA-LNP vaccines elicit higher magnitude of binding antibody responses in adults and children compared to prior infection.

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Introduction: While children infected with SARS-CoV-2 tend to experience milder disease compared to adults, the mechanisms underlying reduced COVID-19 disease severity are poorly understood. We sought to characterize age-related differences in SARS-CoV-2 immunity in response convalescent infection. We hypothesize age-related differences in immunity will also be present in vaccinated individuals.

Objectives: Compare the breadth and magnitude of antibody responses of children and adults after infection with or vaccination against SARS-CoV-2.

Methods: Serum samples were collected one month after SARS-CoV-2 vaccination or one month after SARS-CoV-2 infection. The cohort included 15 convalescent and 16 vaccinated children aged 5-11 years, 20 convalescent and 10 vaccinated adolescents aged 12-17 years, and 8 convalescent and 10 vaccinated adults. A binding antibody multiplex assay (BAMA) panel of 10 variant spike proteins was used to ascertain the magnitude of binding of spike-specific IgG, reported as background corrected mean fluorescence intensity (MFI). An additional BAMA panel of four omicron subvariant spike proteins was used to assess IgG binding breadth to these recent, highly transmissible variants of concern.

Results: Convalescent adults exhibited higher infection-induced binding antibody responses to the ten variants of concern compared to convalescent children and adolescents. The median D614G specific IgG MFI was 9800 in adults, 2438 in adolescents, and 5859 in children aged 5-11 years. A similar trend emerged in the vaccinated cohort in which SARS-CoV-2 vaccination elicited higher antibody responses in adults compared to children and adolescents. For children aged 5-11 years, vaccination elicited higher levels of binding antibodies compared to convalescent children. For example, the median D614G specific IgG MFI was 12,146 in vaccinated children compared to 5,859 in convalescent children. Similarly, the median omicron BA.1-specific IgG MFI was 6032 in vaccinated children compared to 2788 in convalescent children. A similar trend was observed among adolescents and adults in which higher binding antibody responses were observed in vaccinated compared to convalescent adolescents and adults, respectively. Notably, within every age group, vaccinees exhibited higher binding antibody responses against the omicron subvariants BA1.1, BA.2, BA.4, and BA.5 than convalescent individuals.

Conclusions: SARS-CoV-2 vaccination elicited greater binding antibody responses across variants of concern and omicron subvariants in all age groups compared to responses elicited by recent natural infection. Vaccinated adults developed higher semi-quantitative antibody responses than vaccinated children and adolescents. Notably, vaccinated participants across all age groups developed robust antibody responses against all tested omicron subvariants despite receipt of a vaccine targeting the original viral strain. These findings suggest the vaccination remains a viable protective strategy even as new variants circulate.

Role of disrupted cell-specific iron handling in the propagation of kidney fibrosis

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Introduction: Chronic kidney disease (CKD) affects 10-15% of the U.S. population, including more than 200,000 children. Kidney fibrosis is the final pathway common for all individual diseases leading to CKD. Disruption of iron metabolism is one of the systemic complications of CKD. Our recent data (JCI Insight, 2023) indicate that most kidney macrophages are pathologically iron-deficient in kidney fibrosis, and this iron deficiency status induces pro-fibrotic responses of kidney macrophages that propagate fibrosis. However, the mechanism of iron deficiency of kidney macrophages and the cellular iron metabolism in other cell types that participate in fibrosis has not been elucidated in CKD.

Objective: Delineate iron metabolism in the key cell populations that orchestrate kidney fibrosis: kidney macrophages and tubular epithelial cells.

Methods: We analyzed the expression of genes implicated in iron metabolism (FTH1 and FTL: iron storage, ferritin heavy and light chains; TFRC: iron importer transferrin receptor 1, a marker of cellular iron deficiency or overload; and SLC40A1: iron exporter ferroportin) in our whole mouse kidney bulk RNA sequencing dataset, as well as in publicly available single cell/nucleus transcriptomic mouse and human CKD datasets. To validate these transcriptomic analyses, we assessed the expression of the respective proteins in whole kidney (adenine and unilateral ureteral obstruction (UUO) models of kidney fibrosis), as well as in sorted kidney macrophages/monocytes and tubular epithelial cells, which were isolated from digested kidneys using CD11b (after Ficoll centrifugation to eliminate neutrophils) and CD133 magnetic microbeads, respectively.

Results: TFRC was suppressed in whole kidney tissues and the proximal tubules of UUO kidneys compared to those of contralateral kidneys, suggesting iron excess in tubular epithelial cells during fibrosis. In contrast, TFRC was induced in the leukocyte population of human kidneys of patients with autosomal-dominant polycystic kidney disease (ADPKD), consistent with our previous data that revealed cellular iron deficiency of mouse kidney macrophages in fibrosis. FTH1 and FTL genes were consistently suppressed across all datasets in both cell types. However, FtH and FtL protein expression was induced in whole kidney tissues and in sorted kidney tubular epithelial cells and macrophages. Taken together with TfR1/TFRC data, this suggests excessive expression of FtH in macrophages and insufficient in tubular epithelial cells. Expression of kidney ferroportin gene and protein was induced in whole kidney tissues, and SLC40A1 was induced in both cell types in ADPKD human kidneys; similar to ferritin, this induction of ferroportin may be protective in tubular epithelial cells and pathologic in kidney macrophages in CKD.

Conclusions: In kidney fibrosis, cellular iron status varies greatly among different cell types participating in fibrosis. Taken together, our single cell transcriptomic analyses and assessment of protein expression in magnetically sorted kidney cells suggests pathologic iron deficiency in kidney macrophages and iron excess in tubular epithelial cells, both likely propagating kidney fibrosis.

First Reported Case of Pleuropulmonary Blastoma in DICER1-related GLOW Syndrome

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DICER1 is a member of the ribonuclease III family of genes that are involved in generating microRNAs, an important regulator of gene expression. Germline variants in DICER1 are associated with predisposition to several tumors including pleuropulmonary blastoma, cervical embryonal rhabdomyosarcoma, Sertoli-Leydig cell tumors, pineoblastoma, and Wilms tumor. In 2014, an overgrowth syndrome known as GLOW syndrome (Global developmental delay, Lung cysts, Overgrowth, and Wilms tumor) was described and attributed to somatic mosaicism for missense variants in the RNase IIIb domain in DICER1. We report a case of a 4-year-old who presented with pleuropulmonary blastoma and molecular analysis of tumor tissue demonstrated two mosaic DICER1 mutations, c.5113G>A (noted in the 14.8% of the tissue resulting in a missense change in the RNase IIIb domain) and c.4004dupA (noted in the 32.3% of the tissue resulting in a frameshift). Review of the medical history and physical examination demonstrated that the patient had autism spectrum disorder, speech delay, macrocephaly, somatic overgrowth, hepatomegaly and mildly enlarged kidneys noted on imaging. No lung cysts were noted on CT imaging. The DICER1 somatic missense variant, c.5113G>A results in an amino acid substitution of glutamine to glutamate in the RNase IIIb domain of the DICER1 gene, adjacent to two other well-reported missense changes associated with GLOW syndrome (c. 5138 A>T and c. 5125 G>T). Germline testing from our patient is currently underway. We suspect that the missense variant is likely to be found in the germline in a mosaic form similar to the other reported patients with GLOW syndrome, and that the nonsense mutation will be restricted to the tumor tissue as a possible second-hit that gave rise to the pleuropulmonary blastoma, a tumor not previously seen in reported GLOW syndrome patients.

Single-cell RNA-Seq delineates peripheral immune landscape and transcriptional heterogeneity in juvenile dermatomyositis

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Introduction: Juvenile dermatomyositis (JDM) is a potentially life-threatening childhood-onset idiopathic inflammatory myopathy that commonly presents with skin rash and muscle weakness. Although the cause of JDM remains unclear, immune dysregulation in the context of environmental and genetic factors is a key contributor to JDM pathogenesis.

Objectives: Comprehensively investigate single cell phenotypical and molecular determinants associated with JDM and its disease activity from peripheral blood.

Methods: Single-cell profiling using 10X Genomics Chromium single cell 3' V3 protocol was performed on peripheral blood mononuclear cells (PBMCs) from a total of 31 children, including 14 patients with active disease, 9 patients in remission, and 8 demographic-matched healthy controls. After doublets removal and ambient RNA decontamination, raw single-cell gene expression data were further filtered for dying cells, normalized, and corrected for batch effect. Processed single-cell data were then clustered to identify major immune cell populations. To further improve detection and annotation of immune cell subpopulations, subcluster analysis were performed for major immune cell types. Frequencies of immune cell subsets were quantified and compared between JDM and healthy children. Finally, differential gene expression analysis was performed between JDM and healthy children as well as between active and remission patients.

Results: Ten major immune cell populations were defined based on 294,082 single cells from 31 children, including T cells, natural killer (NK) cells, B cells and plasma cells, classical and non-classical monocytes, dendritic cells and pDCs, platelets, and a cluster of proliferative cells. Focused analysis of interferon-stimulated genes (ISG) shows that myeloid cells, NK cells, and proliferative cells in active disease are the predominant source of ISG expression, whereas pDC and plasma cells uniformly express the highest level of interferon receptor alpha across disease groups, which largely resembles global ISG-activation in SLE. 70 immune subpopulations were further characterized by subcluster analysis. Cell proportion analysis revealed that frequencies of most of the monocyte and B cell subclusters are significantly increased in active JDM compared to children with inactive disease or healthy controls, including a CD27+, extrafollicular (CXCR5-) switch memory B cell population that specialized in IGHA2 production. In contrast to a global T cell lymphopenia in active disease, we observed a significant expansion of several CD4+/CD8+ subsets with high ISG expression in total PBMC. When compared within the T cell compartment, several subpopulations, including blood T follicular helper cells, Th2 cells, memory Tregs, and TCF7+/GZMB+ memory CD8+ T cells, among others, had increased frequencies in active disease compared to healthy controls. Interestingly, we also observed subsets of dendritic cells and pDCs overrepresented in patients with inactive disease. Taken together, our study has comprehensively evaluated the peripheral immune compartment at a highly granular level and confirmed JDM as a disease associated with systemic interferonopathy, which also provides tangible leads towards phenotypical underpinning and molecular basis of JDM pathogenesis.

Integrated analysis and enhanced visualization for single cell subcluster analysis using Ragas: applications to pediatric autoimmune diseases

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Introduction: Recent developments in computational tools have provided critical support to investigate transcriptional and phenotypical changes from massive single cell sequencing data. One central problem of single cell data analysis is to accurately cluster hundreds of thousands of cells into their corresponding cell types based on their expression, which becomes even more challenging when dealing with homogeneous cell populations, such as to analyze CD4+ T cells subsets. Subcluster analysis provides a practical solution to improve single cell clustering by first dividing total single-cells into several main clusters that each contains cells from one major cell type, followed by looping through each main cluster and re-cluster cells to obtain refined subpopulations. However, most studies adopted subcluster analysis as an ad hoc procedure to improve the resolution and accuracy of single cell analysis with no existing utilities to optimally integrate or visualize analytical results.

Objective: To develop bioinformatics tools that optimize the integration and visualization of single-cell data from subcluster analysis.

Methods: Developed an open-source R package called Ragas (R Advanced Gallery for Analysis of Subclusters). Ragas offers three major contributions: (1) A unified data structure for single cell RNA-Seq analysis. In Ragas, we define an S3 object called “post integration” (“Pi”) object to coordinate analysis and visualization of single cell data. (2) Subcluster re-projection. A novel re-projection algorithm was developed to project subcluster-level cell embeddings back to main clusters. This is accomplished by combining the K nearest-neighbor graphs from the subclusters and the main clusters, followed by re-ranking the top K interactions for each cell. (3) Enhanced visualization. As an addition to existing Seurat visualization functionality, Ragas provides a convenient interface to generate several useful new/improved single cell plots, including matrix plot, annotated dot plot, stacked violin plot, summarized heatmap, cell proportion plots.

Results: Applied Ragas on single-cell gene expression data from peripheral blood mononuclear cells (PBMCs) of patients with juvenile dermatomyositis (JDM) and Systemic lupus erythematosus (SLE), along with demographic-matched healthy controls. A streamlined pipeline integrating gene expression and cell proportion analysis from main and subclusters were illustrated, which also provided convenient interface to visualize single-cell statistics for multi-group, multi-sample designs. Marker analysis demonstrated clear advantage of the new reprojection algorithm in visualizing homogeneous immune subpopulations at the PBMC level, exemplified by improved separability of markers from memory B cell subsets and CD4+ helper T cells. Additionally, Silhouette index was calculated to quantitatively assess the quality of subclusters, which further confirmed that the cell embeddings generated by the new reprojection algorithm provided more effective delineation of immune phenotypic diversity.

Potential Role of Khdc3 in Small RNA Inheritance and Dysregulation of Hepatic Gene Expression

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Introduction: The field of epigenetic inheritance is rapidly emerging and can potentially provide a mechanistic explanation for the many strongly inherited pediatric diseases that genomics-based approaches have largely been unable to explain. Organisms with mutations in the mammalian germ cell gene Khdc3L/Khdc3 have defective oocyte DNA methylation and abnormal fertility, yet the cellular and molecular function of this gene remains unknown. Previous research in our lab has found that oocytes from Khdc3-null mice have global dysregulation of small RNAs that regulate glucose and lipid metabolism. Given our data shows Khdc3 deficiency is associated with dysregulation of critical lipid and glucose metabolism genes, we next looked in the liver for evidence of metabolic dysfunction.

Objective: Evaluate global differences in hepatic gene expression in WT and Khdc3 knock-out (KO) mice, as well as WT mice generated from WT and Khdc3-null ancestry (WT* mice) and KO mice generated from WT and Khdc3-null ancestry (KO* mice).

Design/Methods: Matings were designed to evaluate for differences in hepatic gene expression in genetically identical mice that have a different mutant ancestral history (Figure 1). RNA-sequencing was then performed on livers from WT, WT*, KO, and KO* mice to evaluate gene dysregulation.

Results: Khdc3 KO mice had 1016 significantly dysregulated genes compared to WT mice (p< 0.05). Comparison of WT* mice with WT mice revealed dysregulated genes (p< 0.05) that significantly overlap with the dysregulated genes in the corresponding KO* mice generated from the same heterozygous parents, suggesting that abnormal gene expression may be driven by inherited epigenetic factors independent of direct genetic effects in the organism (Figure 2 & 3). Gene ontology enrichment for the genes uniquely dysregulated in WT* mice compared to WT mice showed genes involved in long chain fatty acid metabolism and lipid metabolism. Furthermore, WT** mice (generated from a WT* female mouse mated with a WT* male mouse) and WT***mice (generated from a WT** female mouse mated with a WT** male mouse) revealed that similar hepatic gene dysregulation persisted across generations.

Conclusions: Genetically identical mice with different Khdc3 mutation ancestry have different aberrantly expressed genes in their liver, suggesting that non-DNA molecules, such as small RNAs, are inherited at the time of fertilization that influence organ function. Ongoing experiments are aimed at examining small RNAs and DNA methylation in the liver.

Broadly neutralizing antibody (bnAb) escape of HIV-env variants in orally SHIV-infected, ART-treated infant rhesus macaques (RMs)

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Introduction: According to the UNAIDS report of 2022, there are currently 1.7 million children living with HIV worldwide. The current WHO guidelines recommend the initiation of antiretroviral (ART) therapy in all infected children. But only 52% of children living with HIV are on ART and although ART can lead to viral suppression, it does not eliminate the virus reservoir that is established very early following infection. Thus, there is a critical need to develop immune-based strategies to achieve a pediatric HIV cure. In previous studies, the development of autologous virus-neutralizing antibodies and passive immunization with broadly neutralizing antibodies (bnAb) therapy were associated with delay in viral rebound after analytical treatment interruption (ATI). However, viral variants that escape neutralization response limit the therapeutic potential of these strategies. To increase our current understanding of how viral neutralization escape develops, our objective was to map the neutralization escape mutations in orally SHIV-infected infant rhesus macaques (RMs) following ATI with and without therapeutic immunization.

Methods: Infant RMs (n=19) were orally challenged with SHIV.C.CH505 and started on triple ART at 8 weeks post-infection (wpi). Nine animals were immunized with two doses each of SHIV-DNA-rhCD40L at 33 and 37 wpi, SHIV-MVA at 41 and 45 wpi and CH505 Env SOSIP protein at 49 and 57 wpi. At 60 wpi, ART was interrupted and all RMs were monitored for rebound. SHIV.C.CH505 virus neutralization titer in plasma was measured. To assess autologous virus neutralization escape variants, single genome amplification was performed on HIV-Env gene, and Env variants were compared to SHIV challenge stock. Finally, mutations previously associated with bnAb resistance were identified.

Results: Equivalent numbers of RMs from ART and ART+vaccine group developed autologous virus neutralizing antibodies (ART:5/10; ART +vaccine:5/9), and the median titer was ~five-fold higher in the vaccine group (ID50=557) vs. the ART group (ID50=124) RMs, who developed response, although not statistically significant (p=0.4). However, there was no difference in median time to viral rebound post-ATI between ART (18 days) or ART+ vaccine group (14 days) (p=0.16). Individual assessment of HIV-Env sequences from animals who rebounded from both groups, even after development of autologous virus neutralization titers, demonstrated the presence of mutations associated with resistance against CD4bs (D130), V2 (N167) and V3-targeting bNAbs (T746). Interestingly, RMs showed a glycan gain at position 332, which is associated with V3 bnAb resistance, even before ART initiation. No specific differences in patterns of mutation in the HIV-Env region was observed in ART vs. ART+vaccine group.

Conclusions: Using an infant RM model mimicking breast milk HIV transmission and therapy, we identified HIV-Env mutations associated with bnAb escape in ART treated animals, even though no difference was observed in mutation patterns between the ART and ART+ vaccine groups--ongoing studies from our group are focused on mapping bnAb escape upon passive immunization with combinations bnAb therapy in infant rhesus macaques. Identifying specific neutralizing antibody escape-mutations will guide development of future active and passive immunization regimens that will minimize viral escape, leading to sustained drug-free HIV remission in infants.

Hematologic and non-hematologic effects of iron therapy in children with chronic kidney disease: pilot pragmatic clinical trial

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Introduction: Anemia is a common complication of chronic kidney disease (CKD) in children, often associated with functional iron deficiency. Clinical Practice Guideline for Anemia in Chronic Kidney Disease (KDIGO) recommends iron therapy when transferrin saturation (TSAT) < 20% and ferritin < 100 ng/mL. However, iron therapy may adversely affect inflammation, oxidative stress, and bone health in CKD. In this non-inferiority trial, we hypothesized that after children with mild anemia of CKD reach the above TSAT and ferritin cutoffs, iron therapy can be safely postponed for additional 12 weeks without compromising their well-being.

Objectives: Compare physical activity and well-being metrics in children with mild anemia of CKD not receiving iron therapy (no iron therapy group) vs. receiving immediate iron therapy (iron therapy group) as recommended by KDIGO over 12 weeks.

Design/Methods: This is an ongoing randomized open-label pilot pragmatic clinical trial (NCT03991169) of patients with CKD stages II-IV, anemia and iron deficiency as defined by KDIGO. Patients who received iron or ESA therapy within the last 3 months were excluded. Post-randomization, iron therapy group received iron sulfate, 3-6 mg/kg/day, vs. no iron therapy in the no iron therapy group. Physical activity (assessed by PROMIS survey) was the primary outcome. Secondary outcomes included fatigue (assessed by PROMIS), grip strength, muscle mass (bioelectrical impedance analysis, BIA), eating behavior (Child Eating Behavior Questionnaire, CEBQ), and hematologic parameters. Comparisons were performed using $\chi 2$ and t tests.

Results: Of 29 enrolled participants, 17 were randomized: 7 into the iron therapy group, 10 into the no iron therapy group. At randomization, groups had similar CKD severity, hemoglobin, iron status, BMI percentile, physical activity, muscle mass, grip strength, and eating behavior.
After 12 weeks median hemoglobin increased by 0.4 g/dL in the iron therapy group and decreased by 0.4 g/dL in the no iron therapy group. Within iron therapy group, median serum iron increased by 19.0 µg/dL, and median TSAT by 3.9%. The differences in hemoglobin and iron status between the groups did not reach the statistical significance. There were no statistically significant differences in physical activity, fatigue, grip strength, muscle mass, and enjoyment of food between the groups.

Conclusion(s): Postponement of therapy for 3 months did not negatively affect well-being outcomes in children with mild anemia of CKD in a statistically or clinically significant manner. Surprisingly, we also did not find differences in hematologic parameters between the groups. Larger trials are needed to optimize criteria for iron therapy initiation in children with CKD.

Discovering novel inflammatory pathways in the kidney of patients with lupus nephritis

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Systemic lupus erythematosus (SLE) is a clinically complex and molecularly heterogenous autoimmune disease that can affect various organs. Lupus nephritis (LN) is the manifestation of SLE in the kidney and remains one of the most serious SLE conditions, as it affects 80% of pediatric SLE patients. Despite heavy treatment with immunosuppressive drugs, LN still represents a major cause of morbidity and mortality leading to development of end-stage renal disease.

LN is considered a glomerulopathy and its diagnosis relies on histopathological examination of the patients' kidney tissue obtained through a biopsy. This diagnostic method classifies LN patients into six different classes based on morphological integrity of the glomeruli. Despite the acceptance of this clinical classification, little is known about the molecular pathways activated across classes of LN and about the contribution of extraglomerular cells to disease development.

In this work we use renal tissue from well annotated pediatric SLE patients to molecularly profile the kidney of patients with different classes of LN. We employ a multiomic approach that includes spatial transcriptomics, imaging mass cytometry and immunochemical methods.

This study aims to describe novel molecular pathways that contribute to LN and will help to categorize patients for their enrollment into successful clinical trials, paving the way towards the use of precision medicine for the treatment of one of the most serious SLE manifestations.

Exosome-associated IgA as a novel biomarker for Systemic lupus erythematosus (SLE) disease activity

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Systemic lupus erythematosus (SLE) is a lifelong autoimmune disease with a wide range of clinical manifestations. SLE patients who frequently experience periods of heightened disease activity have greater chance of having permanent organ damage. Therefore, biomarkers for SLE disease activity would allow for closer monitoring and preemptive treatment. Exosomes are secreted into plasma by all kinds of live cells, thus have the potential of reflecting immune status. Proteomic analysis was performed on plasma exosomes from 2 dependent cohorts of pediatric SLE patients isolated by a protocol newly developed by a collaborative project with Lyden lab. Among the exosomal proteins that were elevated in SLE patients was immunoglobulin isotype IgA, the level of which was also positively correlated with disease activity. Characterizing single plasma exosome through super resolution microscopy showed that signal from IgA increased dramatically after exosome permeabilization, which indicated that IgA-containing exosomes carried IgA as cargos in the lumen and probably were produced by plasma cells. Consistently, the level of exosomal IgA was positively correlated with the intensity of the plasma cell transcriptomic signature of the matched PBMCs, supporting that IgA-containing exosomes were generated by plasma cells. Ongoing effort is made to deciphering the antigen specificity of exosome-associated IgA and understanding their pathogenic significance.

Plasma IgG responses following acute human cytomegalovirus infection during pregnancy

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Introduction: Human cytomegalovirus (HCMV) is a leading cause of congenital defects and hearing loss in infants exposed during gestation, with an estimated 1 in ~150 of all newborns are born with the infection across the globe. Despite the high prevalence and morbidity, there are no effective vaccines to prevent congenital CMV (cCMV) infection. cCMV occurs both in the absence and presence of pre-existing immunity, yet at a much higher rate following primary infection, indicating partial protective immunity of natural infection. However, what constitutes a protective immune response against cCMV remains elusive.

Objectives: We hypothesize that both neutralizing and Fc-mediated effector antibody responses such as antibody-dependent cellular phagocytosis (ADCP) and cytotoxicity (ADCC), against both structural and non-structural viral proteins contribute to protection against cCMV. To define correlates of protection against cCMV following acute infection during pregnancy, we investigated the humoral immune responses to acute infection in a cohort of 399 women diagnosed with CMV infection during early pregnancy as part of a trial of hyperimmunoglobulin therapy to reduce cCMV infection and disease (NTC01376778).

Methods: Over 200,000 pregnant women were serologically screened for acute HCMV infection prior to week 20 of pregnancy, defined by detection of plasma HCMV-specific IgM and low avidity IgG responses. From this cohort, 399 individuals were identified as acutely-infected with HCMV, and among those, 78 were identified as transmitting cCMV based on urine HCMV screening of the newborns, which were equally distributed among the HIG-treated and untreated groups. A subset of 30 participants were selected based on available sample volume and a pre-existing assessment of HCMV viremia by qPCR to define the specificity of plasma HCMV-specific IgG responses following acute infection during early pregnancy; we are blinded to the transmission status of these participants. Using a binding antibody multiplex assay (BAMA), we tested the IgG binding to a panel of HCMV antigens including the antigenic domains of the main fusion protein (gB) and other surface entry glycoproteins (gH/gL, gH/gL/gO, gH/gL/UL128-UL131), tegument proteins (pp65, pp150), and immune evasins (UL16, UL141).

Results & Conclusions: We validated a comprehensive panel of HCMV antigens to test IgG binding and using a BAMA assay, including a detailed panel of the gB antigenic domains. In our pilot cohort of 30 participants, we detected antibody responses to all antigens tested with the highest responses detected against the entry glycoproteins and the pp150 tegument protein. Among the gB antigenic domains, the highest antibody responses were detected against gB Domain II. For each antigen, binding did not differ by whether participants were viremic at the time the sample was taken. Future work will include additional antibody binding and functional assays with our pilot cohort of 30 participants to further define the acute responses to HCMV infection during pregnancy. With a validated panel of assays, we will propensity score match transmitters (n=78) and non-transmitters (n=156) and assess the binding and functional humoral immune response to determine the natural humoral immune correlates of protection against cCMV following acute maternal infection during early pregnancy.

Improving Screening for Household Food Insecurity in a Pediatric Clinic

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Background: Food insecurity has been recognized as a public health threat in the US due to both the high prevalence of affected families and the significant impact on health outcomes. A 2015 American Academy of Pediatrics (AAP) policy statement supports screening for food insecurity using a validated 2-item screen during primary care visits. Currently in our clinic, a paper screening tool associated with a large multisite research project (WECARE) that aims to improve social determinants of health screening has been used in our clinic to screen for food insecurity using the following question“ Do you always have enough food for your family?” Therefore, we sought to incorporate food insecurity screening into visits by verbally asking whether the family had worries about having enough food and/or not being able to afford buying enough food and link families who identify as food insecure with appropriate resources.

Objectives: The aims of this project were 1) to increase identification of household food insecurity by 50% over a 1- year period and 2) refer at least 75% of families with positive screens to federal and/or local food programs.

Methods: The Model of Improvement was used for this QI project over a 15-month period in a resident-run Medicaid primary care pediatric clinic affiliated with an academic center. The QI team created a driver diagram and interventions were derived from tertiary key drivers (Figure 1). An electronic 2-question food insecurity tool was created based on AAP guidelines. Process, outcome, and balancing measures were collected via electronic medical record review (resident documentation of food insecurity screening, patient referrals, and screening for smoking respectively). To test interventions, residents performed multiple plan-do-study-act (PDSA) cycles. For all charts we reviewed, we analyzed the use of the AAP screen, the WECARE screen, and use of any screen for food insecurity (AAP, WE-CARE, and either AAP or WE-CARE screen). Run charts were utilized to display and analyze the data and run chart rules were applied to detect signal of change.

Results: For the first half-year of the project, median rate of screening using the validated AAP 2-question screen was 10%. Screening rate increased from 10% to 40% after educational interventions with residents were conducted (figure 2A). Median rate of screening using the WECARE screener remained at 50% throughout the QI period (figure 2B). Combining both WECARE and AAP screens resulted in a median screening rate of 65% (figure 2C). Among charts reviewed, 135 patients were screened, 14 of whom screened positive (10% positive screen rate). Of the 14 patients who screened positive, 9 patients (64%) had documentation of resources given.

Conclusion: Encouraging the use of both the WECARE screener and verbally asking the validated AAP screen reached more patients than either intervention alone. While universally administered paper screens are a very effective method to increase screening rate, equipping providers with a validated verbal screen adds an additional layer for those who do not receive or complete forms, or to clarify answers on written forms. Lower than expected positive-screen rates and inconsistent documentation of resources provided to families who screened positive were barriers to evaluating our goal of connecting families in need to resources. This project suggests that educating providers about screening

Improving Provider Comfort Around Emergencies with Technology Dependent Patients and Escalation of Care: A Multidisciplinary Approach through in-Situ Simulation

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Introduction: Increased patient acuity and technology dependence on 6C without increasing education, as well as less exposure to pediatric emergencies leading to provider discomfort with these patients and multiple units with different escalation notification systems lead to missed opportunities for scaled escalation of care on 6C and a serious safety event.
Objectives- as part of a larger QI for escalation of care within the Department of Pediatrics we hoped to achieve the following SMART aim- to improve pediatric unit staff comfort with escalating care by 20% by June 2023.

Methods: Our outcome measures were to track safety events related to safety events monthly as well as evaluate pre and post simulation data. Our process measures were participation in in situ simulations as well as tracking the the number of escalations monthly. One of our interventions was in situ, multidisclipnary simulations. These were held during both day and night shift with similar learning objectives for a 10-month-old with chronic lung disease who develops a tracheostomy obstruction. Our balancing measure was tracking other hospital acquired conditions on 6S and 6C.

Results: 30% of pediatric residents have participated thus far, with 8 6C RNs, 1 PA, 2 RTs and 2 PharmDs. The study is ongoing. Resident confidence has after simulations in activating an escalation of care has increased by 40%. RN and other staff data is pending.

Conclusions: In situ and interdisciplinary simulations can be used to address quality and patient safety needs. By using scheduled conference times for day shift simulations, we were able to capture more learners. Similar simulations can be considered for other types of technology dependence.

A Quality Improvement Initiative to Reduce the Rate of Central Line-Associated Bloodstream Infections Using a High-Risk Bundle Protocol in a Level IV NICU

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Background: Neonatal intensive care unit (NICU) patients are one of the most vulnerable populations for central line-associated bloodstream infections (CLABSIs) due to extended hospitalizations and necessary central line access. CLABSIs are associated with significant morbidity and mortality. We identified risk factors for infants at high-risk for CLABSIs (including femoral line, presence of additional devices, dwell time ≥ 2 weeks, history of thrombus, and history of CLABSI), and created a high-risk maintenance bundle to implement in our level IV NICU.

Objective: The primary aim was to increase the time interval between CLABSI events by 50%, from a center line (CL) of 36 days, by July 2023. The secondary aim was to decrease NICU central line days/1000 patient days present by 10% by July 2023.

Design/Methods: This ongoing QI study utilized the Model for Improvement with a series of sequential interventions. Baseline data were collected from January 2021 to December 2021. Interventions included implementation of central line maintenance guidelines, education of NICU staff, creation of a high-risk bundle protocol, and weekly multidisciplinary device rounds to review adherence to central line maintenance guidelines. Outcome and process measures included the time interval between CLABSI events, central line days/1000 patient days present, and compliance of central line audits. Balance measures included incidence of other hospital acquired conditions (HACs), including unplanned extubations and pressure injuries, as well as burns from chlorhexidine gluconate baths. Statistical process control charts (T-chart, U-chart, and P-chart) were used to display and analyze data. Associates for Process Improvement rules for special cause were applied.

Results: There was no special cause variation in the number of days between CLABSI events (CL = 36), however the time interval since the last CLABSI event has been 203 days to present day. There was an 18% reduction in central line days/1000 patient days present (Fig 2). Since initiation of multidisciplinary device rounds, there was an increase from 95% to 99% compliant central line audits. There were no cases of other HACs, and no burns from initiation of chlorhexidine gluconate baths for those patients who met criteria.

Conclusions: Adoption of standardized practices, including bundles and checklists, combined with targeted multidisciplinary device rounds may lead to a significant reduction in CLABSIs. Future interventions include creation of central line insertion guidelines and an electronic medical record hard stop for central line necessity.

Using spatial transcriptomics to reveal the defects in Alagille syndrome hepatocyte to cholangiocyte reprogramming

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The liver is alone among solid organs in its ability to fully regenerate its mass and function following extensive injury. Mouse studies using genetic reporters to trace the hepatocyte or cholangiocyte (biliary epithelial cell) lineages reveal no evidence of contribution from a reserve stem cell population for hepatic cell homeostasis or liver regeneration. An alternative process to stem cell-mediated regeneration is transdifferentiation or adaptive cellular reprogramming by which a cell changes its identity. Using a mouse model of severe bile duct loss, we have demonstrated that transdifferentiation of hepatocytes to cholangiocytes in the liver can build a biliary system that failed to form during development. The hepatocyte-derived biliary system enables resolution of cholestasis and reversal of fibrosis. Unfortunately, the process of hepatocyte to cholangiocyte transdifferentiation is impaired in patients with Alagille syndrome (ALGS) wherein a characteristic feature is bile duct paucity. Alagille syndrome (ALGS) is a multisystem developmental disorder caused by autosomal dominant mutations in JAGGED1 (95%) or NOTCH2 (1-2%), which encode key components of the Notch signaling pathway. Our single cell transcriptomic data in a Jagged1 haploinsufficient mouse experimental model of ALGS reveals that hepatocytes partially enter the cholangiocyte transcriptional program but are unable to fully implement the cholangiocyte transcriptional program. We are using the Resolve spatial transcriptomic platform to assess 100 transcripts in the liver of both the Jagged1 haploinsufficient mouse experimental model of ALGS and human ALGS patients. Spatial transcriptomics will allow us to assess the spatial relationship of niche and hepatocyte to cholangiocyte transdifferentiating cells and to computationally infer required receptor-ligand interactions as well as transcriptional regulators.

Acute or developmental abrogation of cerebellar nuclei neurons has divergent impact on learning

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Perturbations in cerebellar development can have long-lasting impact on both motor and non-motor functions by affecting the communication between the cerebellum and forebrain. The excitatory cerebellar nuclei neurons (eCN) are the major output neurons of the cerebellum, and while the cerebellar nuclei (CN) are spatially divided into 3 subregions, our understanding of subregion-specific functions is lacking. In addition, the behavioral consequences of developmentally ablating a specific subset of eCN in adulthood remains unknown. In particular, the functional consequences of acute versus developmental abrogation of the posterior medial eCN (mCNp), which strongly innervates non-motor thalamus, has not been fully examined. Here, we used SepW1-Cre mice as a novel approach to target the medial eCN embryonically and in adulthood. Using chemogenetics, we acutely inhibited the adult mCNp and observed selectively impaired reversal learning in a water Y-maze (WYM) test, but not spatial working memory or motor functions. To embryonically ablate the mCNp, we generated Engrailed1/2 conditional knockout mice using SepW1-Cre (SepW1-En1/2 CKOs). Interestingly, SepW1-En1/2 CKOs showed normal reversal learning in the WYM test, spatial working memory, and motor functions. This suggests that although the mCNp is critical for reversal learning in an intact brain, developmentally losing the mCNp leads to compensation by remaining neurons in the cerebellar circuit to confer normal reversal learning. We tested the same behaviors in En1/2 CKOs generated using Atoh1-Cre (Atoh1-En1/2 CKOs), which ablates both the mCNp and posterior interposed eCN (iCNp) in the embryo. Atoh1-En1/2 CKOs showed impaired acquisition and reversal learning in the WYM test, but no changes in spatial working memory. Additionally, Atoh1-En1/2 CKOs showed impaired motor learning, hypolocomotion and a decrease in stride length. These results suggest that the remaining neurons are not sufficient to compensate for a larger development loss of eCN in Atoh1-En1/2 compared to SepW1-En1/2 CKOs. The intralaminar nuclei of the thalamus (ILM) and its major downstream target, the dorsal striatum (DS), are critical for motor, acquisition, and reversal learning. We retrogradely mapped two of the ILM nuclei, centrolateral (CL) and parafascicular (PF), using Fluoro-Ruby in control and Atoh1-En1/2 CKOs. The number of CL- and PF-projecting mCNp and iCNp were greatly reduced in Atoh1-En1/2 CKOs, but no changes were seen in other eCN subregions. We next tested the impact of reduced eCN inputs to the ILM on the activity of DS during reversal learning using c-Fos as a proxy for neuronal activity. Consist with our behavior studies, Atoh1-En1/2 CKOs showed a reduced density of c-Fos+ cells in the DS compared, whereas the SepW1-En1/2 CKOs had a similar density to littermate controls. Our results suggest that the remaining brain regions are capable of compensating for developmental loss of a small subset of eCN necessary for learning, but this compensation has limits when the developmental loss is severe.

Flattening of circadian glucocorticoid oscillations drives insulin-mediated obesity, independently of diet

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Chronic stress and high fat diet can both increase fat mass. To understand potential synergies, here we sought to decouple and define their respective contributions to causing obesity and metabolic dysfunction. To recapitulate stress, such as from jet lag, night shiftwork, or work-life imbalances, we implanted corticosterone pellets in mice which flattened circadian glucocorticoid oscillations while maintaining average circulating glucocorticoids at normal physiologically levels. Within 3 days of either a normal chow or high fat diet, mice experience rapid-onset of hyperinsulinemia and a two-fold increase of all fat mass within 21 days, without the mice eating more. While the glucocorticoid-flattening driven hyperinsulinemia is beneficial in preventing elevated circulating glucose and fatty acids levels, the persistently high insulin levels also cause insulin resistance and a shift in lean to fat mass. The hyperinsulinemia and obesity is reversible under normal chow diet conditions, and we are currently testing whether reversibility is impaired under HFD. Taken together, our results so far argue that stress and diet cause obesity in two independent and mechanistically separable ways, suggesting future therapies for personalized and possibly more effective treatments for obesity and metabolic disease.

Elucidating molecular mechanisms underlying stress-induced hyperinsulinemia

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Introduction: Glucocorticoids (GCs) are steroid hormones that are secreted in a diurnal pattern and affect peripheral tissues to control carbohydrate and fat metabolism. Excess GC exposure is known to cause metabolic dysregulation, and we show that disrupting alone, as in chronic stress, has deleterious metabolic effects and increased obesity. GC flattening in mice also leads to hyperinsulinemia. However, the mechanism by which this occurs remains unknown and is the focus of our further experiments.

Methods: GC flattening (GCF) was achieved by implanting subcutaneous corticosterone pellets in C57BL/6J male mice. Islets isolated through dissection of pancreas and digestion with collagenase.

Experiments: 1) Immunohistochemistry with Ki-67 staining of islets in vitro. 2) Static glucose-stimulated insulin secretion assay (GSIS) on in vitro islets. 3) Transmission electron microscopy (TEM) of beta cells in isolated pancreatic islets. 4) Transfection of primary islets with GCamp6F calcium-sensor and Epacs2-cAMP probes, combined with imaging the islets in vitro using confocal microscope, or combined with transplantation of the islets into the anterior chamber of the mouse eye (ACE) and carrying out in vivo imaging using two-photon microscopy.

Results: 1) Ki-67 expression suggests no difference in proliferation. 2) GSIS demonstrates significant increase in insulin secretion in all conditions (low glucose, high glucose, and KCL) in GCF islets at day 3. 3) Insulin granule docking rate is increased in GCF islets. Results of experiment 4 are pending.

Conclusions: One factor responsible for hyperinsulinemia in GCF mice could be increased insulin granule docking which releases more insulin than in placebo mice. Beta cell proliferation does not appear to play a significant role. We are working on establishing in vitro and longitudinal in vivo system to study insulin-release pathways involving calcium and cAMP and determine processes contributing to hyperinsulinemia.