



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

Pediatrics Research Day 2022

Thursday, June 2, 2022
Griffis Faculty Club
521 E. 68th Street, New York City

Highlighting basic and clinical research in children's health by Weill Cornell Medicine/NewYork-Presbyterian faculty, fellows, residents, medical students, and staff.



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Pediatrics



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Children's Health

Pediatric Research Day 2022 Agenda

Thursday, June 2nd, 2022, Griffis Faculty Club

11:15 am-12:00 pm	<i>Check-in / Grab and Go Lunch</i>
12:00 pm-12:05 pm	Welcome from Sallie R. Permar, MD, PhD
<u>12:05 pm-1:30 pm</u>	<u>Session 1: Infections, Immune Dysregulation, Inflammation</u> Moderator: Irina Matei, PhD
12:05 pm-12:30 pm	Invited speaker: Nick Collins, PhD (20 min/5 min Q&A)
12:30 pm-1:30 pm	Accepted Abstracts (10 minutes/5 min Q&A): Gang Wang, PhD (12:30 pm-12:45 pm) Lei Li, PhD (12:45 pm-1:00 pm) Zurong Wan, PhD (1:00 pm-1:15 pm) Lisa Giulino Roth, MD (1:15 pm-1:30 pm)
1:30 pm-1:45 pm	<i>Break</i>
<u>1:45 pm-2:45 pm</u>	<u>Session 2: Neuroscience and Brain Development</u> Moderator: Barry E. Kosofsky, MD, PhD
1:45 pm-2:10 pm	Invited speaker: Anjali Rajadhyaksha, PhD (20 min/5 min Q&A)
2:10 pm-2:30 pm	Invited speaker: M. Elizabeth Ross, MD, PhD (15 min/5 min Q&A)
2:30 pm-2:45 pm	Accepted Abstract (10 min/5 min Q&A): Zachary Grinspan, MD, MS
2:45 pm-3:00 pm	<i>Break</i>
<u>3:00 pm-4:10 pm</u>	<u>Session 3: Nutrition and Growth</u> Moderator: Amy Tsou, MD, PhD
3:00 pm-3:25 pm	Invited Speaker: Camila R. Martin, MD, MS (20 min/5 min Q&A)
3:25 pm-4:10 pm	Accepted Abstracts (10 min/5 min Q&A): Stephanie Rager, BS (3:25 pm-3:40 pm) Liana Senaldi, MD (3:40 pm-3:55 pm) Matthew Smith-Raska, MD, PhD (3:55 pm-4:10 pm)
<u>4:10 pm-5:00 pm</u>	<u>Career Panel</u> Moderator: Virginia Pascual, MD Panelists: Marianne E. Nellis, MD, MS; Lisa Giulino Roth, MD; Melody Zeng, PhD; Eric J. Mallack, MD
<u>5:00 pm – 7:30 pm</u>	<u>Poster Session and Reception</u>
7:30 pm	<i>Pediatrics Research Day concludes</i>

Presenters and Panelists

Nick Collins, Ph.D.

Assistant Professor of Immunology in Medicine
Joan & Sanford I. Weill Medical College
Jill Roberts Institute for Research in Inflammatory Bowel Disease
Friedman Center for Nutrition and Inflammation
Weill Cornell Medicine

Zachary Grinspan, M.D, M.S.

Interim Chief of Child Neurology; Associate Professor of Pediatrics;
Nanette Laitman Clinical Scholar in Healthcare Policy and Research/Prevention - Women's Health; Associate Professor of Population Health Sciences
Weill Cornell Medicine

Barry E. Kosofsky, M.D., Ph.D.

Professor of Pediatrics; Professor of Pediatrics in Radiology; Professor of Neurology
Professor of Neuroscience; Horace W. Goldsmith Foundation Professor of Pediatrics
Weill Cornell Medicine

Lei Li, Ph.D.

Senior Bioinformatics Analyst
Gale and Ira Drukier Institute for Children's Health
Weill Cornell Medicine

Eric J. Mallack, M.D., M.B.E.

Assistant Professor of Pediatrics; Assistant Professor of Neurology;
Director, Leukodystrophy Center
Weill Cornell Medicine

Camila R. Martin, M.D., M.S.

Chief, Newborn Medicine;
Assistant Professor of Pediatrics (Interim)
Weill Cornell Medicine

Irina Matei, Ph.D.

Assistant Professor of Immunology Research in Pediatrics
Weill Cornell Medicine

Marianne E. Nellis, M.D., M.S.

Associate Professor of Pediatrics;
John D. & Lili R. Bussel, M.D. Associate Professor in Pediatric Hematology
Weill Cornell Medicine

Virginia Pascual, M.D.

Director, Gale and Ira Drukier Institute for Children's Health;
Gale and Ira Drukier Director of Children's Health Research, Administration;
Ronay Menschel Professor of Pediatrics; Professor of Pediatrics
Weill Cornell Medicine

Stephanie Rager, B.S.

Medical Student, Weill Cornell Medical College
Weill Cornell Medicine

Anjali Rajadhyaksha, Ph.D.

Professor of Neuroscience, Brain and Mind Research Institute; Professor of Neuroscience in Pediatrics, Weill Cornell Medicine; Associate Dean Program Development, Weill Cornell Graduate School of Medical Sciences

M. Elizabeth Ross, M.D., Ph.D.

Nathan Cummings Professor and Head, Laboratory of Neurogenetics and Development; Director, Center for Neurogenetics
Chair, Neuroscience Graduate Program
Weill Cornell Medicine

Lisa Giulino Roth, M.D.

Associate Professor of Pediatrics; Associate Professor of Pediatrics in Medicine; Associate Professor of Pathology and Laboratory Medicine
Weill Cornell Medicine

Liana Senaldi, M.D.

Instructor in Pediatrics
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Matthew Smith-Raska, M.D., Ph.D.

Friedman Family Foundation Clinical Scholar in Newborn Medicine; Assistant Professor of Pediatrics
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Gang Wang, Ph.D.

Postdoctoral Associate in Pediatrics
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Melody Zeng, Ph.D.

Assistant Professor of Immunology in Pediatrics
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Oral Presentation Abstracts

Tumor-derived extracellular vesicles and particles systemically promote Kupffer cell-induced fatty liver formation and drug-metabolizing dysregulation

Presenting Author: Gang Wang (Postdoc, gaw2012@med.cornell.edu)

Authors: Gang Wang¹, Jianlong Li¹, Linda Bojmar¹, Haiyan Chen¹, Zhong Li², Robert E. Schwartz³, Haiying Zhang¹, and David Lyden¹

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²Duke Proteomics and Metabolomics Shared Resource, Duke University School of Medicine, Durham, NC, USA

³Division of Gastroenterology and Hepatology, Department of Medicine, Weill Cornell Medicine, New York, NY, USA

Introduction: Cancer is a systemic disease. In addition to cancer cells metastasizing to and growing within distant organs, and thereby disrupting their physiological functions, primary tumor cells also release soluble factors (such as proteins, cytokines and hormones) and extracellular vesicles and particles (EVPs) into the circulation that can establish pre-metastatic niches at future sites of metastasis. Tumor-secreted factors may also have the capability to systemically reprogram the functions of cells in multi-organs that are otherwise metastasis-free

Objectives: Investigate the role of tumor-derived extracellular vesicles and particles in functional reprogramming of the liver in the context of cancer.

Methods: Liver tissues from tumor-bearing mice, cancer patients, tumor-EVP educated mice were subjected to RNA-Seq, metabolomics and lipidomics MS, and BODIPY staining. Precision cut liver slices (PCLS) from tumor-bearing mice were subjected to drug-metabolizing assay. PCLS from naïve mice after treatment of tumor-EVPs were subjected drug-metabolizing assay.

Results: We identified that tumor-derived EVPs package multiple fatty acids, such as palmitic acid, and target Kupffer cells, thereby upregulating tumor necrosis factor alpha (TNF α) via Toll-like receptor 4. This in turn promoted a proinflammatory microenvironment leading to fatty liver formation and downregulated metabolic pathways, such as fatty acid metabolism and oxidative phosphorylation. Tumor implantation or pre-treatment with tumor EVPs decreased the expression of cytochrome P450 genes and attenuated drug metabolism in mice. Increased fatty livers and decreased cytochrome P450 genes were also observed in tumor-free livers in cancer patients.

Conclusions: Our results highlighted the role of tumor EVPs in dysregulating hepatic functions and its potential to serve as therapeutic targets along with Kupffer cell-induced TNF α inhibition to prevent fatty liver formation and enhance anti-cancer chemotherapy.

Comprehensive and multimodal analyses of B cell and T cell receptor repertoire using VGenes

Presenting Author: Lei Li (Senior Bioinformatics Analyst, lel4003@med.cornell.edu)

Authors: Lei Li¹, Jiayi Sun¹, Jenna J. Guthmiller², Yanbin Fu¹, Siriruk Changrob¹, Joshua JC McGrath¹, Nai-Ying Zheng¹, Min Huang¹ and Patrick C. Wilson^{1*}

¹Gale and Ira Drukier Institute for Children's Health, Weill Cornell Medicine, New York, NY 10021, USA.

²Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA.

Introduction: Recent advances in single-cell sequencing technology have enabled large scale B cell receptor (BCR) and T cell receptor (TCR) repertoire profiling with simultaneous measurement of transcriptome expression, cell surface protein expression, and antigen-binding at the single-cell level, allowing efficient screening of antigen-specific monoclonal antibodies and in-depth study on mechanisms of B and T cell immunity. However, the constantly evolving single-cell technology leads to rapid growth in both volume and complexity of single-cell sequencing data, posing significant challenges on downstream data analysis and interpretation.

Objectives: We aim to design an integrated pipeline for BCR/TCR analysis that 1) is efficient and capable of processing large-scale datasets; 2) is versatile and compatible with multiple modalities of single cells; 3) is user-friendly to biologists without any computational background.

Methods: Using state-of-the-art databasing and developing technology, we built an efficient, extendable, robust pipeline workflow. By introducing and developing computational algorithm MS to identify BCR/TCR clones and functional annotations, analyze BCR sequence patterns and correlations, and select candidates for antibody cloning and expression, we have made this pipeline versatile, comprehensive, and friendly to biologists.

Results: We propose VGenes, an integrated graphical platform for multi-modal analysis of single-cell-RNA-sequencing (scRNAseq)-based BCR/TCR repertoire. In VGenes, the user-friendly graphical user interface (GUI) allows users to easily visualize, analyze, and edit the BCR/TCR sequences of each individual B/T cell. Along with BCR/TCR profiling, VGenes enables multi-model analysis of the cellular transcriptome, surface protein expression, and antigen specificity at the single-cell level. After the analysis, VGenes provides users with cloning strategies to synthesize and express specific monoclonal antibody candidates selected for downstream experiments. Together, VGenes is a user-friendly, highly efficient, integrated analysis platform that facilitates research of B cell and T cell immunity.

Small Extracellular Vesicles and Particles (EVPs) as novel biomarker for Systemic lupus erythematosus (SLE)

Presenting Author: Zurong Wan (Postdoc, zuw4001@med.cornell.edu)

Authors: Zurong Wan¹, Jinghua Gu¹, Uthra Balaji¹, Seunghee Hong¹, Pernille Lauritzen¹, Jeanine Basich¹, Cynthia Smitherman¹, Marina Lima Silva Santos¹, Cristy Stager¹, Marina Ohouo¹, Simone Caielli¹, Haiying Zhang¹, Irina Matei¹, David Lyden¹ and Virginia Pascual¹.

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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 and organ involvement would allow for closer monitoring and preemptive treatment. Small Extracellular Vesicles and Particles (EVPs) are secreted into plasma by all kinds of live cells, thus have the potential of reflecting immune status and organ inflammation. By performing proteomic analysis of small EVPs from more than 60 SLE patients isolated by a protocol newly developed by a collaborative project with Lyden lab, we discovered multiple potential biomarkers for disease activity and lupus nephritis. Among these proteins, EVP-associated IgG level decreased in SLE patient with active lupus nephritis. More interestingly, we identified a cluster of 6 proteins whose EVP expression level correlate with each other, preferentially presented in patient with disease activity, and most of which specifically expressed by intestine. Ongoing effort is made to verify these findings with a validation cohort, evaluate the predicting power of these potential biomarkers and understanding their biological significance.

Molecular evolution of classic Hodgkin lymphoma revealed through whole genome sequencing of Hodgkin and Reed-Sternberg cells

Presenting Author: Lisa Giulino Roth (Faculty, lgr2002@med.cornell.edu)

Authors: Francesco Maura, Bachisio Ziccheddu, Jenny Z. Xiang, Bhavneet Bhinder, Federico Abascal, Kylee H. Maclachlan, Kenneth Wha Eng, Manik Uppal, Feng He, Wei Zhang, Qi Gao, Venkata Yellapantula, Vicenta Trujillo-Alonso, Sunita Park, Matthew Oberley, Elizabeth R

The rarity of malignant Hodgkin and Reed Sternberg (HRS) cells within a classic Hodgkin lymphoma (cHL) biopsy limits the ability to study the genomics of cHL. To circumvent this, our group has previously optimized fluorescence-activated cell sorting to purify HRS cells. Here we leveraged this method to report the first whole genome sequencing landscape of HRS cells and reconstruct the chronology and likely etiology of pathogenic events prior to the clinical diagnosis of cHL. We identified alterations in driver genes not previously described in cHL, a high activity of the APOBEC mutational signature, and the presence complex structural variants including chromothripsis. Children and young adults age 0-40y had an increased mutational burden compared to older adults. We found that the high ploidy observed in cHL is often acquired through multiple, independent large chromosomal gain events including whole genome duplication. Evolutionary timing analyses revealed that RAG-mediated structural variants, driver mutations in B2M, BCL7A, GNA13, and PTPN1, and the onset of AID-driven mutagenesis usually preceded large chromosomal gains. The study provides the first temporal reconstruction of cHL pathogenesis.

Phenylbutyrate for STXBP1 and SLC6A1 mutations - a pilot clinical trial

Presenting Author: Zachary Grinspan (Faculty, zag9005@med.cornell.edu)

Authors: Zachary Grinspan¹, Kerry Gao¹, Jaqueline Burre¹, Jennifer Cross¹, Natasha Basma¹, Alexandra Lamar², Scott Demarest², and M Elizabeth Ross¹

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Introduction. Mutations in STXBP1 and SLC6A1 each can lead to developmental delay and epilepsy. Preclinical data for both STXBP1 and SLC6A1 indicate that phenylbutyrate can increase protein function. We launched a pilot clinical trial to administer glycerol phenylbutyrate to 20 children (10 with each disorder).

Methods. This is a single treatment group, multiple-dose, open-label, pilot study to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of glycerol phenylbutyrate in children with STXBP1 encephalopathy and SLC6A1 neurodevelopmental disorder at two centers, Weill Cornell Medicine and Children's Hospital Colorado. Each participant is enrolled for 14 weeks (4 weeks baseline, 8 weeks of drug exposure, and 2 weeks follow-up), with an option for 1 year extended use. The primary endpoints are safety and tolerability. Exploratory clinical outcomes include seizure burden, EEG abnormalities, abnormal movements, quality of life, development, behavior, sleep, and caregivers' qualitative experiences. Planned enrollment is 15 at Weill Cornell Medicine, 5 at Colorado Children's Hospital. We report interim seizure frequency outcomes.

Results. We have enrolled 15 children here at Weill Cornell (9 with STXBP1 and 6 with SLC6A1). 12 have received glycerol phenylbutyrate, 10 have completed the core 14 weeks of the study, and 9 requested extended use. Of the 10 that have completed the core 14 weeks of the study, 8 had seizures. Of these eight, all have had reduction of seizure frequency including four with greater than 90% reduction (two with STXBP1 and two with SLC6A1). Common side effects include somnolence and a honey-like body odor. There has been one serious adverse event probably related to the study drug - a child was admitted for metabolic acidosis, which resolved upon discontinuation of the medication.

Conclusions. Early clinical experience suggests phenylbutyrate therapy is a promising approach for seizure control in STXBP1 and SLC6A1. Full results are pending trial completion.

Inherent Sex Differences in the Neonatal Gut Microbiome and Gastrointestinal Inflammatory Immune Response

Presenting Author: Stephanie Rager (Medical Student, slr4001@med.cornell.edu)

Authors: Jenny Jin, Katherine Z. Sanidad, Aparna Ananthanarayanan, Jeffrey M. Perlman, and Melody Y. Zeng

Introduction: Male sex is an independent risk for major NICU morbidities, but the etiology of these sex differences is unclear. Previous in vitro studies in our lab suggest that preterm female stool may elicit enhanced inflammatory response in macrophages.

Objective: To investigate sex differences in the gut microbiome and immune response in preterm neonates

Methods: Neonates born between 33 and 41 weeks and admitted to our level IV NICU or Well Baby Nursery without receiving antibiotics were enrolled. Fresh stool was collected and frozen at -80C. 24 neonatal stool samples were sent for preliminary bacterial 16S sequencing and lipidomic analysis. 3-week-old germ-free mice (n=28) were gavaged with fecal slurries from 3 preterm males, 3 preterm females of comparable gestational and chronological ages, or left as controls. After 2 weeks, mice were provided with 1% dextran sodium sulfate (DSS) water to induce colitis, then sacrificed 8 days later. Flow cytometry, qPCR, and histologic analysis were performed using colonic and splenic tissue. Additionally, 3 groups of mice (female stool gavage, male stool gavage, controls) were infected orally with *Citrobacter rodentium*, a murine homolog of Enteropathogenic and Enterohemorrhagic *E. coli*; bacterial dissemination to the spleens and livers was assessed 24 hours later.

Results/Conclusions: Preliminary 16S analysis revealed increased abundance of *Bacteroides* in term female neonates compared to term males and late preterm females. Late preterm females had increased abundance of *Staphylococcus* compared to late preterm males. DSS-exposed mice gavaged with female stool had significantly ($p < 0.05$) less weight loss at day 8 compared to mice gavaged with male stool and control mice. Mice gavaged with female stool also had significantly longer colons compared to mice gavaged with male stool. Flow cytometry revealed decreased colonic neutrophils in mice gavaged with female stool. Additionally, mice gavaged with female stool had reduced dissemination of *Citrobacter rodentium* to the spleens and livers. Together, these data suggest inherent differences between male and female neonatal microbiomes and a protective role of female gut bacteria. Future studies will focus on identifying beneficial strains enriched in female neonates and determining how they confer protection from inflammatory and infectious stimuli.

The Effects of Prenatal Corticosteroid Exposure on Small RNA Expression in Germ Cells

Presenting Author: Liana Senaldi (Faculty, lis9110@med.cornell.edu)

Authors: Liana Senaldi^{1,2}, Nora Hassan¹, Jinghua Gu², Uthra Balaji², and Matthew Smith-Raska^{1,2}

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Background: Epigenetic abnormalities in sperm and oocytes are undervalued drivers of inherited disease. Studies have revealed that ancestral exposures can affect descendants' phenotypes by inheritance of non-DNA molecules through the germline. Small RNAs are being increasingly recognized as critical components of this process, most importantly by affecting gene expression.

Little is known about the long-term effects of glucocorticoid exposure on developing germ cells in humans. Women at risk for preterm birth are routinely treated with antenatal steroids to promote fetal lung development and reduce preterm infant morbidity and mortality. Synthetic glucocorticoids cross the placenta and can influence the developing fetus by generating epigenetic changes that accumulate in an individual's germ cells and affect disease risk in descendants.

Objective: The objective of this study was to evaluate the small RNA profile of developing oocytes exposed to in utero dexamethasone in a mouse model. We also evaluated how the small RNA expression in response to in utero dexamethasone is affected in a *Khdc3* knockout mouse (*Khdc3* is a gene that we believe is an important regulator of small RNAs in response to exposures).

Methods: Oocytes were dissected from F1 generation dexamethasone exposed and non-exposed mice. RNA was isolated using a Trizol-based method and then cleaned of all organic impurities using a magnetic bead-based approach. Small RNA libraries were generated and sequenced to a depth of 30 million reads per sample. Libraries were generated using the Takara SMARTer small RNA-Seq kit and sequenced on an Illumina NovaSeq system. Bioinformatics processing and analysis were performed to compare the quantity and types of small RNAs between dexamethasone exposed and non-exposed mice.

Results: The developing germ cells in the F1 generation exposed to in utero dexamethasone have abnormal expression of small RNAs, including microRNAs, piwiRNAs, and tRNA fragments ($p < 0.01$). We further demonstrate that a *Khdc3* knockout mouse fails to induce oocyte small RNA expression in response to in utero exposure to dexamethasone ($p < 0.001$), suggesting that this gene is an important regulator of the small RNA response to exposures.

Conclusion: This study provides new perspectives on the mechanisms and factors involved in inheritance of diseases in response to ancestral exposures across multiple generations.

Khdc3 Regulates Oocyte Small RNAs that Control Hepatic Lipid and Glucose Metabolism, with Implications for the Epigenetic Inheritance of Obesity and Metabolic Disease.

Presenting Author: Matthew Smith-Raska (Faculty, mrs7001@med.cornell.edu)

Authors: Liana Senaldi^{1,2}, Nora Hassan¹, Uthra Balaji², Junghua Gu², and Matthew Smith-Raska^{1,2}

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INTRODUCTION: An individual's diet has a significant effect on the risk of obesity and metabolic disease in their descendants (even if they consume a healthy diet); this process is mediated largely by epigenetic factors such as small RNAs that are inherited via the germ cells. This phenomenon has been demonstrated convincingly in rigorous animal models and observational human studies, yet the cellular and molecular processes that regulate diet-driven epigenetic changes to the germ cells remains completely undefined. Based on preliminary data, we are studying the mammalian gene *Khdc3* as a critical regulator of oocyte small RNAs, with potential implications for the intergenerational inheritance of obesity and metabolic disease.

OBJECTIVES: Our goal was to examine the small RNA profile of *Khdc3*-deficient oocytes, and determine how dysfunctional small RNA expression affects gene expression in the oocyte. We are also interested in how abnormal small RNA expression in the oocyte affects the inheritance of disease risk across generations.

METHODS: We developed and utilized a novel protocol to examine the small RNAs in the oocytes of wild type and *Khdc3*-deficient mice. This was supplemented by global transcriptome analysis of *Khdc3*-deficient oocytes.

RESULTS: *Khdc3*-deficient oocytes demonstrated significant dysregulation of multiple micro RNAs, piwi RNAs, ribosomal RNAs, and tRNA fragments. While the molecular functions/targets of many of these small RNAs are unknown, two significantly upregulated miRNAs (miR-99b and miR-125a) are critical regulators of hepatic lipid and glucose metabolism, with important roles in the development of obesity and diabetes. These results are corroborated by the observation that *Khdc3*-deficient oocytes have abnormal expression of multiple genes important for hepatic lipid and glucose metabolism. Collectively, this data suggests that *Khdc3* affects expression of small RNAs in the oocyte that regulate transcriptional programs critical for normal hepatic glucose and lipid metabolism. We are currently examining the effects of a Western-style high fat diet on small RNA expression in *Khdc3*-deficient oocytes, and how this affects the intergenerational inheritance of obesity and metabolic disease that has been well described in wild type mice.

Poster Presentation Abstracts

Rates, Risk Factors and Consequences of High Blood Pressure Among Adolescents in Sub-Saharan Africa: A Systematic Review and Meta-Analysis

Presenting Author: Rachel Abramson (Medical Student, rha4001@med.cornell.edu)

Authors: Mussa Kelvin Nsanya, Godfrey Kisigo, Andy Hickner, and Robert Peck

There is a growing number of reports on high blood pressure (BP) among adolescents in low- and middle-income countries, including in Sub-Saharan Africa (SSA). Overweight and obesity among adolescents is relatively higher in developed countries and is the major driver of high BP. In contrast, adolescents in SSA are leaner and the contribution of obesity to the rising prevalence of hypertension remains relatively low. In addition, risk factors such as unhealthy diet and physical inactivity are common, but similarly their contribution to the rising burden of hypertension remains low. There is a need to explore the contribution of endemic (non-traditional) risk factors. High BP among adolescents in high income countries has been shown to track to adulthood, and is associated with end-organ damage, including left ventricular hypertrophy, chronic kidney disease and cognitive decline; similar data among adolescents in SSA is lacking.

The objective of our systematic review is to add knowledge on epidemiologic patterns of primary high BP among adolescents (age 10-19) in SSA by determining rates, risk factors and clinical consequences of high BP. Risk factors and health consequences of high BP have not been explored in this population in prior systematic reviews. We conducted a systematic literature search in multiple databases (Ovid Medline, Embase, Google scholar, Web of Science and African Index Medicus). Key search terms were: adolescent, arterial hypertension and sub-Saharan Africa; both key words and medical subject heading (MeSH) terms were used.

Preliminary results show that hypertension among adolescents in SSA ranges from 1% - 19% and pre-hypertension ranges from 8.7% - 35% (this variation may be partially explained by differences in study population age ranges). Identified risk factors include overweight and sedentary lifestyle, but also malnutrition, female sex (in contrast to male sex in developed countries), low parental socio-economic status, and malaria infection in the mother during pregnancy. Studies describing consequences of high BP in adolescents were limited. There is a need for more studies looking at adolescents as a separate population, calculating rates of high BP rather than only treating BP as a continuous variable, and investigating consequences of high BP in SSA.

Lymphatic dysfunction in systemic lupus erythematosus

Presenting Author: William Ambler (Fellow, amblerw@hss.edu)

Authors: Madhavi Somaraji, JiHyun Sim, Ethan Seltzer, Noa Schwartz, Camila Carballo, Scott Rodeo, Raghu Kataru, Jinyeon Shin, Babak Mehrara, and Theresa Lu

Background: Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease in which patients frequently have photosensitivity or exacerbated responses to ultraviolet radiation (UVR). In addition to eruption of cutaneous lesions, exposure to UVR can induce systemic disease flare. The lymphatic system is the route in which the skin communicates to the local immune system by draining fluid, soluble molecules, and cells from the skin to the draining lymph node. Dysfunctional lymphatics have been shown to exacerbate acute inflammation and to alter draining lymph node responses including loss of tolerance to self-antigens. We hypothesize that lymphatics are dysfunctional in patients with SLE and contribute to abnormal responses to UVR.

Objectives: Our goals are to investigate lymphatic function in SLE patients and murine lupus models. Further we aim to assess the immunologic consequences of manipulating lymphatics.

Methods: We assessed lymphatics in SLE patients by performing immunohistochemistry of skin biopsies. Murine lupus models were used to assess and manipulate lymphatics in vivo. MRL/lpr and an indubitable model using chronic epicutaneous application of imiquimod on B6 mice were used. Lymphatics were manipulated using a novel mouse model (PTENLEC) with enhanced lymphatics (B6 PTEN^{fl/fl} Flt4ERT2) and by manual lymphatic drainage. Lymphatic function were investigated in murine lupus models with an Evan's blue assay. Skin and draining lymph node responses were investigated by flow cytometric analysis after tissue digestion.

Results/Conclusions: Patients with SLE have dilated cutaneous lymphatics, which is a marker of poor lymphatic flow. Lupus prone mice had reduced lymphatic drainage compared to controls after UVR. Photosensitivity was reduced when lymphatic drainage was improved in PTENLEC model or by MLD. PTENLEC mice also demonstrated reduced immune activation of the draining lymph node compared to controls. Improving lymphatic flow decreased plasmablast survival, in part by upregulating fibroblast reticular cell (FRC) CCL2. This data suggests that lymphatic dysfunction contributes to cutaneous photosensitivity as well as changes in the draining lymph node in SLE mouse models. Future studies will assess for further evidence of lymphatic dysfunction in SLE patients, assess the etiology of lymphatic dysfunction, and will expand on the immunologic consequences of manipulation of lymphatic flow.

Mapping CD4 T cell heterogeneity in systemic lupus erythematosus at the single cell level

Presenting Author: Preetha Balasubramanian (Postdoc, prb2011@med.cornell.edu)

Authors: Preetha Balasubramanian¹, Uthra Balaji¹, Cynthia Smitherman¹, Lynnette Walters^{2,3}, Jeanine Baisch¹, Tracey Wright^{3,4}, Jinghua Gu¹, Simone Caielli¹, and Virginia Pascual¹

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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 patient with high disease activity (DA) regulatory CD4⁺ T cells (Treg) expressing naïve markers are expanded although their pathogenic function is still not elucidated. Given the heterogeneity of CD4⁺ T cell in the peripheral blood of the SLE patients, it is important to characterize this compartment at the single cells level. Indeed, we have performed single cell RNA sequencing (sc RNA-seq) of purified CD4⁺ T cell from the blood of 12 children with SLE and 8 matched healthy controls for a total of 195,763 cells. This analysis confirms the presence of two transcriptionally distinct Treg populations; one characterized by an activated phenotype (CD45RA⁻ CD25^{hi}) and one with a more naïve phenotype (CD45RA⁺ CD25^{low}). The latter one is significantly expanded in SLE patients. We have also found the expansion, in SLE patients, of a CD4⁺ T cell subpopulation expressing high levels of interferon inducible genes (ISGs). Our study reveals distinct differences in the CD4⁺ T cell compartment between children with SLE and to healthy control and might help to identify new potential pathogenic CD4⁺T cell subsets.

YAP and TAZ extend clonal expansion during adipogenesis.

Presenting Author: Henri Berger (Graduate Student, heb4001@med.cornell.edu)

Authors: Henri Berger, Sanjeev Sharma, and Mary Teruel

Obesity and diabetes are major health issues, however not all obesity is equally unhealthy. De novo adipogenesis, the ability to produce more fat cells in response to weight gain has been shown to mitigate the worst metabolic disorders. Unfortunately, most obese adult humans have limited capacity to produce new fat cells and have unhealthy hypertrophic fat tissue. Identifying the factors that regulate the number of fat cells produced could lead to novel therapies to improve metabolic health.

During adipogenesis, progenitor cells respond to hormonal stimulation and enter clonal expansion. During this phase, they undergo a variable number of cell divisions, allowing them to produce more fat cells. Clonal expansion ends when PPAR γ , the master regulator of adipose function, reaches a critical threshold during G1 and the cells differentiate.

Recent studies show that whole-body overexpression of YAP in mice leads to increased fat mass and proliferation of adipocyte progenitors, independent of caloric intake, suggesting that YAP regulates adipogenesis. We hypothesize that modulation of YAP/TAZ activity controls the duration of clonal expansion during adipogenesis, and thus the number of fat cells produced.

We leverage live single cell time-lapse microscopy in fluorescently labelled reporter cell lines to simultaneously monitor cell-cycle progression and differentiation commitment in the same preadipocyte. We use acute regulation of YAP activity using a Dox inducible promoter paired with an auxin inducible degron tag for temporal control of YAP expression. We also use siRNA knockdown and small molecule inhibitors and show that activation of YAP/TAZ with a Lats1/2 inhibitor increases proliferation of progenitors during adipogenesis and reduces the fraction of differentiated cells.

Overexpression of constitutively active YAP or TAZ results in a hyper-proliferative phenotype marked by increased EDU incorporation, and severely blocks differentiation. Furthermore, double knockdown of YAP and TAZ accelerates adipocyte commitment. Together, these data suggest that downregulation of YAP and TAZ is critical for exit from the clonal expansion window and terminal commitment.

Suppression of Ferritin Heavy Chain in Myeloid Cells Improves Anemia of Chronic Kidney Disease.

Presenting Author: Chantalle Campbell (Research Technician, chc4015@med.cornell.edu)

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Introduction: Anemia is a common complication of chronic kidney disease (CKD), which greatly reduces the quality of life in children with CKD. In iron-naïve children with CKD, serum ferritin paradoxically correlates with the severity of anemia. However, the causative role of ferritin alterations in the development of anemia in CKD has not been established. It is not known whether ferritin heavy chain (FtH), a ferroxidase that converts Fe^{2+} to Fe^{3+} , is implicated in anemia of CKD, specifically within myeloid cells which specialize in iron handling.

Objectives: Elucidate the role of myeloid FtH in the development of anemia of CKD using a mouse model.

Methods: We used a high adenine diet to induce CKD in 8 week old wild type (WT) and myeloid-specific (LysM-Cre) Fth-knockout (KO) mice. Blood was collected at euthanasia for complete blood counts and serum separation. Data were analyzed using ANOVA; p-values <0.05 were considered statistically significant.

Results: Myeloid Fth deletion was confirmed by immunoblotting of bone marrow derived macrophages obtained from Fth KO and WT mice. Myeloid Fth deletion did not affect kidney function, kidney histology, or hemoglobin levels in control mice without CKD. Kidney function was severely reduced in both WT and KO mice with CKD compared to respective control mice without CKD as assessed by blood urea nitrogen and serum creatinine. Kidney histology revealed extensive tubulointerstitial fibrosis in CKD mice. Spleen iron stain showed increased number of iron-loaded macrophages in WT CKD but not in KO CKD mice compared to their respective controls. Anemia in Fth KO CKD mice was significantly less severe than in WT CKD mice.

Conclusions: Myeloid-specific deletion of Fth improved anemia in young mice with CKD. This likely indicates that ferroxidase activity of FtH is pathologically induced in CKD, which may result in excessive intracellular storage of iron in myeloid cells thus limiting iron availability for erythropoiesis.

Impact of immunological imprinting by seasonal coronaviruses in COVID-19 patients

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Most humans had been previously exposed to other antigenically distinct common seasonal human coronaviruses (hCoVs) before the COVID-19 pandemic. Immune cross-reactivity between SARS-CoV-2 with other hCoV have been proven to exist. Understanding of immunological memory to seasonal hCoV in SARS-CoV-2 infected subjects are required to conclude whether pre-existing to seasonal hCoV or de novo response to SARS-CoV-2 could shape protective immunity against COVID-19 disease. In this study, we analyzed serum antibody and memory B cell against spike, nucleoprotein (NP), and receptor-binding domain (RBD) of SARS-CoV-2 and other four seasonal hCoVs in COVID-19 patients with variety range of infection severity. We found that subjects with a more severe SARS-CoV-2 infection exhibit greater antibody response to the S2 of SARS-CoV-2 and OC43. Moreover, we revealed that serology bias responses of beta-hCoV-spike and alpha-hCoV-NP are associated with disease severity in acute patients and convalescent donors. Additionally, hCoV spike, RBD and NP-specific memory B cells are stably maintained over 150 days post-COVID-19 symptom onset. We then asked how these pre-existing memory B cell and cross-reactive antibodies shapes SARS-CoV-2 immunity. We used single-cell sequencing to profile hCoV-reactive B cells with oligo-tagged antigen baits approach. SARS-CoV-2 spike-specific cells were enriched in the memory compartment of acutely infected and convalescent patients while large proportion of seasonal hCoV-reactive antibody-secreting cells were identified in severe acute subject which possessed highly mutated variable genes, implying preexisting immunity. Finally, cross-reactive monoclonal antibodies targeting S2 were non-neutralizing and non-protective in vivo. These findings reveal evidence of immunological imprinting that determine the antibody profile to COVID-19 patients that might be related to the disease outcome.

Quantitative Assessment of Lung Structure in Neonates using Magnetic Resonance Imaging (MRI) in the neonatal intensive care unit (NICU)

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Introduction: Bronchopulmonary dysplasia (BPD) is a serious morbidity of preterm birth¹. The diagnosis of BPD is clinical² and does not address the underlying heterogeneous pathophysiology of the lung compartments: airway, parenchyma, and pulmonary vasculature³. Historically, the neonatal lung has been poorly imaged. Chest X-ray is limited, and CT is rarely used due to ionizing radiation exposure⁴. Novel techniques in lung MRI offer the potential to non-invasively assess structural lung abnormalities in BPD.

Objective: To evaluate quantitative MRI measures of lung structure in infants with and without BPD

Methods: Infants admitted to the NICU with a clinically prescribed brain MRI were considered for inclusion in this IRB-approved study. Parents provided consent to add a 15-minute lung MRI to the brain protocol without sedation. Image analysis was performed with 3DSlicer and a regional growing algorithm. Maximum change in tracheal cross-sectional area (CSA), as defined by the % change between the maximum and minimum CSAs above the carina, and lung volumes / body surface area (BSA) were calculated.

Results: 16 infants (2 term, 14 preterm) have been enrolled to date. 8 infants had BPD based on the 2018 NIH definition (1 grade I, 5 grade II, 2 grade III). One was excluded due to motion artifact. One infant with a tracheostomy was excluded from tracheal analysis. None of the infants had tracheomalacia. All had a maximum change in tracheal CSA <50% (n=14, mean 33.2% $\pm$ 8%), the clinically accepted threshold for defining tracheomalacia^{5,6}. Mean lung volume/BSA was 593 mL/m² $\pm$ 248 (n=8) for infants with BPD, and 529 mL/m² $\pm$ 59 (n=7) for without BPD (P=0.50). Infants with Grade III BPD had the largest volumes (mean 841 mL/m², n=2). Infants with Grade II BPD had the smallest volumes (mean 483 mL/m², n=5).

Conclusions: Quantitative MRI may be useful in assessing lung structure in infants with BPD, specifically to evaluate for tracheomalacia and parenchymal disease. Variations in volume/BSA in infants with BPD may reflect heterogeneity in underlying pathophysiology. Larger volumes may reflect air trapping, whereas smaller volumes may reflect fibrosis. More studies will be needed to understand how quantitative lung MRI should be interpreted in the context of BPD.

Augmentation of autologous virus neutralizing antibodies following DNA/MVA/SOSIP vaccination strategy in oral SHIV.C.CH505-infected infant rhesus macaques

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Introduction: Despite the availability and effectiveness of anti-retroviral therapy (ART), ~150,000 children are newly infected with HIV each year, of which >50% of infections occur postnatally, during breastfeeding. The establishment of HIV reservoir immediately after infection remains a primary barrier to achieve HIV cure. While early antiretroviral (ART) treatment initiation reduces the size of viral reservoir, it is not curative. In low resource settings, where most new pediatric HIV infections occur via breastfeeding, early ART initiation is not feasible due to delay in diagnosis. Therefore, an immune-based intervention in combination with ART would greatly benefit postnatal HIV infection in children via breastmilk transmission. In this study, we used previously established infant rhesus macaque (RM) oral SHIV infection model to determine the impact of novel active vaccination regimen in combination with ART to augment autologous virus neutralizing antibodies and to delay viral rebound following ART interruption.

Method: Infant R MS (n=20) were orally infected with SHIV.C.CH505 and treated with ART starting at 8 weeks post infection (wpi). Half of these animals (n=10) were immunized with SHIV 1086C DNA/MVA adjuvanted with CD40 ligand and boosted with CH505 SOSIP/Alum. Treatment was interrupted at 60 wpi and animals were followed for viral rebound.

Results: Plasma viral load (PVL) peaked at 2 wpi (PVL range = $30 - 1.22 \times 10^7$ RNA copies/ml) and became undetectable upon treatment initiation (8 wpi). Upon ART interruption, virus rebounded in 9 of 10 animals in the ART group and in 8 of 9 animals in combination therapy group, with no significant difference in time to viral rebound. Antibodies against HIV CH505 gp120 and MN gp41 were detected longitudinally in both groups of macaques. In addition, antibodies against 1086C gp120 and uncleaved pre-fusion optimized (UFO) Env trimer were detected following DNA/MVA/SOSIP immunization and boosting with CH505 SOSIP/Alum did not boost the plasma antibodies binding to CH505 gp120. Interestingly, combination therapy not only augmented the autologous virus neutralizing antibodies in the plasma (Median ID50 Combination=2098; median ID50 ART=46.5), but also increased the frequency of animals developing such response (100% autologous neutralizing antibody positive), compared to ART only group (50% autologous neutralizing antibody positive).

Conclusion: ART with DNA/MVA/SOSIP immunization leads to augmentation of the autologous neutralizing antibodies compared to ART alone. However, the augmentation of the autologous plasma neutralization is insufficient to delay viral rebound likely due to escape variants. Assessment of viral evolution that confers immune escape to plasma neutralization warrants further investigation.

Retrospective Analysis on the Use of Aprepitant in Cannabinoid Hyperemesis Syndrome

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Introduction: Cannabinoid hyperemesis syndrome (CHS) is a functional gastrointestinal disorder presenting as recurrent paroxysms of severe nausea and vomiting, similar to the episodes exhibited by cyclic vomiting syndrome (CVS), in patients with excessive cannabinoid use. As cannabinoid use has increased among adolescents over the past decade, hospital admissions for CHS have also increased. Given the rising prevalence, cost, and healthcare burden associated with CHS, it is necessary to identify novel agents for treatment. Aprepitant is a neurokinin-1-receptor antagonist frequently used in the treatment of chemotherapy induced vomiting and CVS. Given the demonstrated benefit of aprepitant in these disorders, it may prove to be useful in the treatment of CHS.

Objectives: This study aims to (1) describe the use of aprepitant in pediatric patients with CHS, (2) explore the characteristics of patients who received aprepitant, (3) quantify the use of alternative antiemetics when aprepitant is administered, and (4) define the number of emergency room visits and hospitalizations for patients who received aprepitant.

Methods: A retrospective chart review of the NYPQ inpatient EMR was completed to identify patients aged 10-22 who were seen in the ED or admitted due to symptoms of CHS from 2018-2020.

Results: 26 patients were seen for CHS and 6 received aprepitant. 73% of all patients were 18 years or older. Among the patients who received aprepitant, 2 patients had three or more hospital visits prior to receiving aprepitant, demonstrating high symptom burden in this cohort. Five of 6 patients who received aprepitant had no further visits to our institution following aprepitant use. 4 of 6 patients who received aprepitant required less total doses of antiemetics post-aprepitant (49%) compared to pre-aprepitant, and the remaining 2 patients required no additional doses of antiemetics post-aprepitant. There was an absolute reduction in the number of doses of ondansetron (46%), metoclopramide (83%), and other antiemetics (14%). The other antiemetics included lorazepam, diphenhydramine and haloperidol.

Conclusions: The use of aprepitant in CHS is advantageous by decreasing reliance on alternative antiemetics and decreasing future hospital visits. By using aprepitant, patients were able to receive fewer doses of medications associated with dose limiting side effects such as QTc prolongation, sedation, and addictive potential.

Mesothelin Targeting Chimeric Antigen Receptor Therapy for Pediatric Acute Myeloid Leukemia

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Introduction: Despite advances in targeted therapies, one in four children with AML will relapse. There remains an urgent unmet need for improved therapies. A substantial subset of patients (25-35%) of pediatric AML patients aberrantly express mesothelin (MS LN), a cancer discriminating immunotherapeutic target associated with increased aggressiveness.

Objectives: We aimed to assess the efficacy of a second generation MS LN targeting chimeric antigen receptor (CAR) T cell with a 28-zeta costimulatory domain (M28z) against pediatric AML. We hypothesized that M28z CAR T cells have antitumor efficacy against MS LN+ AML.

Methods: AML human cell line, Kasumi-1, was retrovirally transduced with an SFG vector to stably express MS LN and GFP-firefly-luciferase fusion protein. In vitro efficacy at varying effector to target (E:T) ratios was evaluated using luciferase assays. 3×10^6 MS LN+ tumor cells were injected systemically via tail vein into NSG mice to produce clinically relevant mouse models with high disease burden. 5×10^5 M28z CAR T cells (E:T ratio of 0.2:1) were injected systemically at day 7, when a high leukemic burden was visualized in bone marrow. Overall Survival was analyzed via Kaplan-Meier estimate and compared between groups using a log-rank test. Secondary outcomes included tumor burden as measured noninvasively with bioluminescent imaging (BLI) and flow cytometry of bone marrow/spleen.

Results: In vitro assays demonstrated >50% MS LN+ tumor cell lysis with E:T ratios as low as 1:1, without toxicity against MS LN- control cells. M28z treated mice with an E:T ratio of 0.2:1 demonstrated significantly prolonged survival (median 62 days, $p < 0.0007$). Control mice were euthanized at median 38 days for progressive leukemic burden, as measured with BLI and flow cytometry after necropsy. Disease burden was eradicated by 7 days after treatment in the M28z group. By 62 days, the M28z did not show signs of acute cytokine release (no weight loss, reduced activity, or malaise).

Conclusions: Preliminary data demonstrates that M28z CAR T cells specifically target MS LN+ leukemic cells in vitro at low E:T ratios. M28z CAR T cells at an E:T ratio of 0.2:1 significantly prolonged median survival in cell line derived xenograft murine models with a high burden of AML.

Inhibition of sphingolipid synthesis decreases SARS-CoV-2 infection in human airway epithelial cells

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Introduction: Asthma in children is linked to viral respiratory infections for both pathogenesis and exacerbations, but SARS-CoV-2 rarely leads to severe diseases. Variations at the 17q21 locus, in particular the minor T allele of single nucleotide polymorphism rs7216389, are strongly linked to childhood asthma and viral triggers for wheezing. We observed in a cohort of children with and without asthma in New York City, shortly following the first surge of COVID-19, that the asthma risk allele of rs7216389 decreased the risk for SARS-CoV-2 infection. Sphingolipid synthesis is inhibited in asthma children with the rs7216389 risk allele. Functionally, the risk allele of rs7216389 increases ORMDL3, which is a negative regulator of serine palmitoyltransferase (SPT), the rate-limiting enzyme for sphingolipid synthesis. We hypothesized that decreased sphingolipid synthesis could affect the infectivity of SARS-CoV-2.

Objectives: We investigated if pharmacological inhibition of SPT decreases SARS-CoV-2 viral entry and replication in the human airway epithelial cells in vitro.

Methods and Results: The human airway epithelial cell line Calu-3 and the primary human nasal epithelial cells (HNE) fully differentiated at the air-liquid interface were treated with 1 μ M myriocin, the SPT inhibitor, to inhibit sphingolipid synthesis. Calu-3 and HNE were pretreated with myriocin for 5 hours and 22 hours respectively, then infected with the recombinant vesicular stomatitis virus pseudotyped β -galactosidase-NanoLuc luciferase that contains SARS-CoV-2 spike protein (rVSV- β -galactosidase-NanoLuc-SARS-CoV-2-Spike). The viral entry was assessed by luciferase activity in the cells 24 hours after the infection. Myriocin-treated cells showed decreased rVSV- β -galactosidase-NanoLuc-SARS-CoV-2-Spike uptake in both cell types. To assess viral replication of SARS-CoV-2, human pluripotent stem cells-derived lung airway organoids (hPSC-AWOs) were pretreated with 0.1 μ M and 1 μ M myriocin for 18 hours, and then infected with SARS-CoV-2 (WA/1 strain) at 0.2 M.O.I. The infected cells were collected 48 hours after the infection and viral N sgRNA and E RNA were quantified by RT-qPCR. The viral RNA in the myriocin-treated hPSC-AWOs were decreased in a dose-dependent manner.

Conclusions: Inhibition of sphingolipid synthesis decreases SARS-CoV-2 viral entry and replication in human airway epithelial cells. Modulation of sphingolipid synthesis could be a therapeutic target to inhibit SARS-CoV-2 infection.

Gastrointestinal microbiome composition is associated with reduced risk of postnatal Simian/Human Immunodeficiency virus (SHIV) acquisition in infant rhesus macaques.

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While antiretroviral therapy (ART) is effective in reducing mother-to-child transmission of HIV, ~150,000 infants are infected each year, owing to poor maternal ART adherence and new infections occurring during breastfeeding. Interestingly, despite repeated daily HIV exposure during breastfeeding, only 10% of infants of untreated infected mothers acquire the virus. Yet, there is little understanding of the mechanisms of reduced HIV acquisition in these exposed infants. While maternal antibodies and innate antiviral components in breast milk play crucial roles in reducing the risk of oral HIV acquisition, the association of infant gastrointestinal (GI) microbiome and viral infection has not been extensively studied. Here, we investigated whether specific GI commensals can delay the risk of oral HIV acquisition.

We leveraged archived rhesus macaque (RM) samples, in which nursery-reared infant R MS (n=12/group) were immunized with either an HIV regimen or RSV vaccine (control) and challenged orally with SHIV1157ipd3N4QNEgp120. Stool samples were collected pre-challenge and 16S rRNA gene sequencing was performed followed by analysis using DADA2 pipeline. A novel microbe-phenotype triangulation platform was used to identify bacterial taxa associated with decreased HIV susceptibility. Furthermore, the bioinformatically identified bacterial taxa were assessed for their ability to inhibit HIV-NLGI using TZM-bl-based reporter cell line assay.

While HIV immunization did not delay time to SHIV acquisition in infant R MS, animals from both groups required multiple challenges to get infected, implying the role of host and environmental factors in oral viral acquisition. The stool 16S rRNA sequence analysis from the two groups independently identified similar bacterial taxa associated with reduced risk of viral acquisition. Using cellular model, we identified 3 strains of *Lactobacillus gasseri* that can suppress HIV replication in a range of 52-68%, confirming previous ex vivo findings. Additionally, we identified 2 bacterial species within the family Lachnospiraceae, *Clostridium immunis* and *Ruminococcus gnavus* that can suppress HIV replication by 78% and 87%, respectively.

Our data indicate that specific commensals in GI microbiome can interfere with HIV acquisition in infant R MS. Mechanistic understanding of HIV-infected host cells with identified bacteria will guide modulation of the microbiome of infants, thereby reducing their susceptibility to postnatal HIV acquisition.

A simulation and case-based curriculum to improve pediatric residents' knowledge of and confidence in identifying child abuse

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Introduction: Child abuse and neglect are leading public health problems, annually affecting 700,000 U.S. children, and result in significant morbidity and mortality. Studies suggest that physicians often lack knowledge and confidence in identifying cases of abuse and neglect, and frequently receive no formal training, despite being mandated reporters. Medical education research cites lack of clinical exposure to cases of abuse and neglect as frequently cited reasons for inadequate training, highlighting the need for interactive curricula specifically.

Objective: To implement and assess a novel interactive pilot curriculum on child abuse and neglect for residents at a pediatric residency program.

Methods: Nine pediatric residents from all years of training participated in a two-part pilot curriculum on child abuse and neglect. The first session was a 1-hour case-based lecture that reviewed features of physical abuse, sexual abuse, neglect, and medical child abuse. The second session was a high-fidelity simulation of a child with abusive head trauma, which required residents to elicit a social history from a caregiver while performing medical management. During the debrief, residents were asked to justify whether the case should be reported to child protective services. Residents completed a pre- and post-participation knowledge test and confidence survey.

Results: A Wilcoxon signed-rank test was used to analyze pre- and post-knowledge test scores. Pre-test median score of 80 (IQR: 60-80) was higher than post-test median score of 60 (IQR: 60-100) but the difference was not statistically significant ($p = 0.3$). A Likert analysis was performed to detect changes in confidence scores. The median confidence score for each survey question remained unchanged or improved by one level (on a 5-point Likert scale) when comparing pre- and post-participation responses. Qualitative feedback included statements such as "this should be part of the regular curriculum" and "more simulations would be useful".

Conclusion: We created and implemented a novel interactive pilot curriculum on child abuse and neglect for pediatric residents including a case-based lecture and high-fidelity simulation. Quantitative analysis of changes in resident knowledge and confidence was limited by sample size. Participant feedback will be used to refine the curriculum in future cycles on a larger sample.

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

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Authors: Mackensie Gross, Caitlin Williams, Stephanie Langel, Maria Blasi, and Sallie Permar

Rotavirus causes ~200,000 deaths/year in children <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. Rotavirus is highly transmissible and is spread by fecal-oral route, aerosolized droplets, contaminated food and water, and fomites. Lower vaccine efficacy in LMICs has been attributed to malnutrition, gastrointestinal tract health, and high enteric disease burden. Additionally, placentally-transferred IgG and breast milk-transferred secretory IgA interfere with vaccine responses in infants and children. Human rotavirus replication is restricted in the mouse model, likely due to the presence of protective commensal bacteria in the murine gut. We hypothesize that antibiotic therapy will enhance human rotavirus replication due to the subsequent decrease of protective commensal gut bacteria.

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

Three routes of pup antibiotic exposure will be tested: intrauterine, breast milk, and direct oral administration. Pups will be challenged with 10^6 FFU of Wa strain human rotavirus after antibiotic exposure and tested for presence of rotavirus antigen via ELISA and viral RNA via RT-qPCR. Observation of clinical signs of disease such as diarrhea and intestinal pathology will determine disease progression and severity.

In an initial 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). However, there was no discernable intestinal tissue pathology due to rotavirus infection compared to that of uninfected mouse pups. Since disruption/depletion of normal murine flora likely enhances human rotavirus replication, ongoing experiments will determine whether pre-treatment of 2-7 day old SV129 mouse pups with antibiotics (gentamicin, metronidazole, and ampicillin) via intrauterine, passive transfer in breast milk, and direct oral route could enhance the pathogenicity of human rotavirus intestinal disease, including diarrhea/weight loss, antigen detection, and intestinal pathology, providing potential improvement upon existing models to study infant human exposure to rotavirus and vaccines.

Development and evaluation of a computable phenotype for multisystem inflammatory syndrome in children

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Introduction: Following acute SARS-CoV-2 infection, some children develop multisystem inflammatory syndrome in children (MIS-C). Understanding of MIS-C and its long term consequences would be enhanced by tools to accurately identify affected children in large electronic health records (EHR) and administrative databases.

Objectives: To develop and validate a computable phenotype to accurately identify patients with multisystem inflammatory syndrome in children (MIS-C) using electronic health records.

Methods: We performed a retrospective analysis of electronic health record data at a single center, NewYork-Presbyterian/Weill Cornell Medical Center. We manually reviewed the charts of pediatric patients reported to the New York State Department of Health for suspected MIS-C to create a gold standard cohort. We then reviewed an International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10) diagnosis codes associated with each child to develop a working computable phenotype. As new cases accrued, we tested and refined the algorithm over time, including the addition of medication information. We report our final algorithm and its performance.

Results: The final computable phenotype using billing codes, M35.81 (MIS-C) or [(U07.1 (COVID-19) or Z20.822 (exposure to COVID-19)) and (M30.3 (Kawasaki disease) or I40.0 (infective myocarditis))], had sensitivity of 98% (detected 42 of 43 cases) and positive predictive value of 61% (42 of 69 had MIS-C). Adding administration of intravenous corticosteroids and/or immune globulin to the computable phenotype kept sensitivity of 98% while improving positive predictive value to 84% (42 of 50 had MIS-C).

Conclusion: We developed a computable phenotype for MIS-C that performed well with good positive predictive value and excellent sensitivity.

Linkage to Care Intervention to Improve Post-Hospital Outcomes Among Children with Sickle Cell Anemia in Tanzania: A Pilot Study

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Background: Of the 300,000 infants born with sickle cell anemia (SCA) yearly, 75% are born in sub-Saharan Africa. Approximately 50%-80% of these children will die before adulthood. A previous observational study in Tanzania showed that children with SCA have a 20% chance of dying in the year following hospitalization. We conducted a pilot study to determine the effectiveness of a linkage to care intervention with social workers to improve 12-month post-hospital mortality for Tanzanian children with SCA.

Methods: The study was conducted at Bugando Medical Center (BMC) in Mwanza, Tanzania from June 2018 - October 2019. Eligible participants were children with SCA hospitalized at BMC. Children enrolled into the study underwent a social worker intervention that consisted of five meetings over a 3-month period after hospitalization. The primary outcome was mortality. Secondary outcomes were clinic attendance and cumulative rate of readmission. Outcomes were measured at 3, 6 and 12 months after hospitalization. Comparison was done with a historical cohort. We also interviewed families and healthcare workers to identify factors influencing linkage to care.

Results: Fifty-nine children were enrolled. One hundred percent completed all five social work sessions. The post-hospital mortality at 12 months was 4/59 (6.7%). This was significantly lower than the 19.2% mortality observed in the historical cohort (adjusted Hazard Ratio (aHR) 0.26, 95% CI 0.08 to 0.83; $p=0.023$). Clinic attendance improved from 45.6% in the historical cohort to 93.2% in the interventional cohort (aHR 7.05, 95% CI 2.39 to 20.77; $p<0.001$), and the cumulative rate of readmission was 50.8% in the historical cohort compared with 70.4% in the interventional cohort (aHR, 1.17; 95% CI, 0.73-1.87; $P = 0.543$).

Sixty-two interviews were performed. Key factors identified as influencing linkage to care included lack of education, traditional health beliefs, lack of home/social support, far distance to clinic, failure to remember appointments, and low perceived need for SCA services for child.

Conclusion: Implementing a social worker intervention during the first 3 months after hospitalization for children with SCA may improve clinic attendance and reduce post-hospital mortality. Further research should be directed at interventions to reduce barriers to linkage to care.

Effect of bioactive sphingolipids on airway reactivity

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Introduction: Asthma, the most common chronic respiratory disease in children, has a strong genetic predisposition through variations at the 17q21 locus. There is increasing evidence that sphingolipid metabolism is altered in childhood asthma and is linked to airway hyperreactivity. Still, there is a gap in knowledge on how sphingolipid metabolism is functionally linked to asthma pathogenesis.

Objectives: Based on recent findings, children with asthma and 17q21 risk alleles have an increased ratio of the sphingolipid mediator sphingosine 1-phosphate (S1P) to sphinganine 1-phosphate (Sa-1P; exclusively generated by de novo synthesis) in blood cells, similar to the S1P/Sa-1P ratio in blood and lungs of sphingolipid-deficient mice. S1P is known to contract airway smooth muscle cells via activation of S1P receptors, relevant to asthma. However, little is known about the function of Sa-1P. We hypothesize that Sa-1P can dampen by counteracting agonist-associated airway contractility of S1P.

Methods: To understand the effect of sphingolipid mediators on airway reactivity, alone and in combination, we evaluated the effects of S1P and Sa1P on small airway contractility in precision-cut lung slices (PCLS). Airway contraction was quantified by visualization of a small airway (200-300 μ m) lumen on PCLS using video phase-contrast microscopy.

Dose-dependent responses of S1P and Sa-1P alone, at various ratios, as well as before and after methacholine, were assessed.

Results: We found that S1P induces a strong dose-dependent small airway contraction in contrast to Sa-1P, which only induces minimal contraction.

Adding Sa-1P to S1P reduced the S1P airway contractility. This effect was achieved at half the concentration of S1P, suggesting that Sa-1P competes with S1P at S1P-receptor binding.

Methacholine-induced contractions were not affected by either S1P or Sa1P.

Conclusion: Sa-1P maybe act as a functional antagonist on S1P receptors on airway smooth muscle that may be relevant for airway hyperreactivity in asthma.

An Evaluation of HCMV mRNA/LNP Vaccine-induced IgG Subclass and Fc Receptor Binding Strength in Humans

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Human cytomegalovirus (HCMV) is the most common infectious agent causing congenital infection, severe abnormalities in infants, and life-threatening disease in immunocompromised individuals. HCMV vaccine development has been a “Tier 1 priority” of the National Academy of Medicine for over twenty years. One candidate is the mRNA vaccine developed by Moderna, mRNA-1647, which encodes the HCMV gB and pentameric complex and is encapsulated in a lipid nanoparticle and has been shown to elicit strong binding and neutralizing antibody responses in preclinical models. mRNA-1647 was evaluated in both seronegative and seropositive vaccine recipients in a phase I clinical trial (NCT03382405), including an administration of three doses of 180 µg at 0, 2, and 6 months. We utilized the Binding Antibody Multiplex Assay to measure the binding strength of vaccine-elicited gB and PC-specific IgG antibodies to Fc receptors (FcRs), important for antibody effector functions, and to characterize the gB and PC-specific IgG subclasses 1, 2, and 3 in vaccinee sera before vaccination and 4 weeks after the final dose. We found that for both gB-specific and PC-specific IgG antibodies, IgG1 and IgG3 subclasses predominated in seronegative and seropositive vaccinees, before and after vaccination. mRNA vaccination elicited gB-specific IgG 1, 2, and 3 subclass antibodies that bound to all tested Fc receptors in seronegative vaccine recipients, while IgG binding trended towards an increase in seropositive vaccinees. For mRNA vaccine-elicited PC-specific antibody responses, seronegative and seropositive mRNA-1647 vaccinees demonstrated an increase in IgG1 subclass antibodies and FcR binding, indicating a boost upon vaccination. Notably, we found that the PC-specific IgG subclass responses and their binding strength to FcRs, was significantly higher than that of gB-specific IgG responses in seronegative vaccinees. Interestingly, in seropositive vaccinees, the binding strengths of gB-specific and PC-specific IgG after vaccination were similar to all FcRs except FcRn, in which the PC-specific IgG binding strength was higher. Collectively, our data indicates that the mRNA vaccine induced potent HCMV glycoprotein-specific IgG1 and IgG3 antibodies and FcR binding capabilities, particularly for PC-specific antibody responses, suggesting Fc-mediated antibody functionalities can be mediated by mRNA HCMV vaccine-elicited antibodies.

Comparison of Humoral Immune Responses Elicited by Glycoprotein B Subunit and mRNA/LNP HCMV Vaccines in Human

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Human CMV (HCMV) is the leading infectious cause of birth defects and neurodevelopmental deficits and is also responsible for a significant disease burden in immunocompromised individuals. Hence, an effective HCMV vaccine is urgently needed to prevent infection and HCMV-associated disease in those populations. The MF59-adjuvanted glycoprotein B subunit vaccine (gB/MF59, Sanofi) demonstrated ~50% efficacy in preventing infection in multiple phase 2 trials. However, adequate efficacy was not achieved for further clinical development. The mRNA-1647, an mRNA vaccine candidate against HCMV developed by Moderna, consists of six mRNAs encapsulated in lipid nanoparticles. Five of these mRNAs encode the HCMV pentameric glycoprotein complex (PC), and one mRNA encodes the full-length gB. gB/MF59 and mRNA-1647 were administered in 3 doses at 0, 1, or 2, and 6 months to adolescent girls ages 12-17 (gB/MF59, dose 20 μ g) and adults 18-49 (mRNA-1647, dose 180 μ g), respectively. The gB-specific immune responses to the two different vaccines were assessed utilizing a suite of antibody binding and cell-based functional assays. Our data demonstrated that mRNA-1647 is highly immunogenic in vaccine recipients by inducing potent PC and gB-specific IgG responses. Vaccine-induced IgG1 and IgG3 were dominant subclasses in both cohorts, but gB/MF59 vaccinees developed overall higher gB-specific IgG responses than the mRNA-1647 cohort. Comparable responses were noted when the two vaccine groups were compared for neutralizing antibody (NAb) responses in fibroblasts (Towne strain). NAb titers positively correlated with IgG responses to Antigenic Domain 4 in gB/MF59 cohort, a known NAb target. Finally, the gB-transfected cell-binding IgG level, an identified immune correlate of protection in gB/MF59 vaccinees, was higher in gB/MF59-vaccinated than mRNA-1647-vaccinated individuals. Additionally, gB/MF59 vaccinees showed higher gB-specific IgG Fc receptor (FcR) binding strength than the mRNA-1647 cohort except for equivalent binding to Fc γ 1A, potentially linked to vaccine-induced IgG Fc-mediated functionality like ADCP. This system serology approach to comparing the humoral immune responses elicited by clinically-tested HCMV vaccine platform can guide the rational design of more effective HCMV vaccines.

Priming of the immune system with CD4bs-precursor germline-targeting SOSIP immunogens to induce a more robust anti-HIV humoral immune response in infant rhesus macaques.

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Introduction: The HIV/AIDS pandemic is still an urgent problem despite HIV being discovered nearly 50 years ago. A vaccine is key to preventing the spread of the AIDS-causing virus. A pediatric vaccine might be especially salient given the unique pediatric immune landscape more amenable to specific immune outcomes necessary to preventing HIV transmission. One such vital outcome is the relatively quick induction of broadly neutralizing antibodies (bNAbs). Human infants have been shown to develop these highly protective antibodies at a much faster rate and at a much higher frequency than adults (Goo et al, 2014). Immunizing infants prior to adolescence, a period of increased risk of exposure to HIV, provides a much longer time window for immunization, should a successful HIV vaccination require several years to induce protective antibodies in the vaccinee. Infant rhesus macaques (R MS) and juvenile R MS are relevant models of vaccine development in humans.

Objectives: We aim to compare the humoral immune responses of infant R MS to those of juvenile R MS sequentially immunized with germline-targeting immunogens to evaluate the immunogens'™ ability to induce anti-HIV CD4-binding site (CD4bs) neutralizing antibodies (NAbs).

Methods: We evaluated immunogen efficacy by measuring the binding ability (ED50) of plasma antibodies isolated from immunized infant R MS (n=5) and immunized juvenile R MS (n=4) via ELISA and evaluated the titers of NAbs via an HIV-1 neutralization assay. Both groups of R MS were primed with BG505 germline-targeting (GT) SOSIP Trimers, modified to target na⁺ve B cells that are precursors to CD4bs NAb-producing mature B cells. Both groups were subsequently boosted with BG505.664 wild-type (WT) SOSIP Trimers to shepherd the further development of these precursor B cells into these mature B cells. Both SOSIP immunizations (50ug) were formulated with a 3M052-SE adjuvant (10ug).

Results/Conclusions: Infant R MS developed higher antibody binding specific to the GT SOSIP immunogen compared to those of their juvenile counterparts. Furthermore, 3 out of 5 infant R MS developed CD4bs precursor NAbs; antibodies that are ultimately precursors to highly protective bNAbs. These results demonstrate that the sequential immunization of infant R MS induces a more effective immune response compared to those of juvenile R MS .

SARS-CoV-2 in stool but not nares of newborns of mothers with COVID-19 during pregnancy

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Introduction: In utero transmission of SARS coronavirus 2 (SARS-CoV-2) has not been fully investigated. Negative nasal PCR tests in newborns born to mothers with COVID-19 during pregnancy do not exclude the possibility of SARS-CoV-2 present in other tissues, such as the intestine. Intestinal reservoirs of SARS-CoV-2 have been found in recovered adults, suggesting persistent intestinal viral accumulation.

Objective: To investigate potential evidence of in utero transmission of SARS-CoV-2 in the stool of newborns born to mothers with COVID-19 infection during pregnancy.

Methods: We investigated stool from 1 day to 2 months of age from 14 newborns born at 25-41 weeks whose mothers had COVID-19 during pregnancy. Newborns were admitted at delivery to the NICU or newborn nursery of our urban academic hospital from July 2020 to May 2021. A comparison group of 30 newborns had similar GAs and were born to mothers without COVID-19 during pregnancy. SARS-CoV-2 RNA was quantified with quantitative PCR using primers against SARS-CoV-2 envelope protein and non-structural protein 14 (NSP-14), spike protein with ELISA, and inflammatory cytokines interleukin-6 (IL-6) and interferon- γ (IFN- γ) elicited by stool homogenates in mouse bone marrow macrophages. This study was IRB approved with parental consent.

Results: Despite negative nasal PCRs from all newborns, viral RNAs and spike protein were detected in the stool of 11 out of 14 newborns as early as the first day of life (range 0-2 months, Figure 2A, 2B). Stool RNA and spike protein levels increased over time in 2 and 4 newborns, respectively (Figure 3A, 3B). Stool homogenates from all newborns elicited elevated inflammatory cytokines, IL-6 and IFN- γ , from mouse macrophages (Figure 1). Most newborns were clinically well except for one death from gestational autoimmune liver disease and one with necrotizing enterocolitis.

Conclusions: These novel findings suggest risk of in utero SARS-CoV-2 transmission to the premature and term fetal intestine during gestation despite negative postnatal nasal PCRs. It is unclear if the presence of viral RNA and protein within the gut microbiome represents active virus in newborns with clinical hospital courses typical of their gestational age in 12 out of 14 cases. However, increasing levels of viral RNA and protein over time suggest replication in some infants, and their gut microbiome induced inflammation in mouse models. The presence of SARS-CoV-2 RNA and spike protein in the intestines of newborns may potentially impact development of the gut microbiome and the immune system and should be further investigated.

Implementing Social Determinants of Health Screening in the Inpatient Setting

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Introduction/Background: Social determinants of health (SDoH) significantly impact healthcare utilization. SDoH screens have been implemented widely in outpatient settings. Despite studies indicating that SDoH screening tools are needed during inpatient hospitalizations, a paucity of data in this setting exists. Reported barriers to inpatient screening are lack of time, resources and a standardized inpatient screening tool. By screening for SDoH in the inpatient setting, medical teams can identify unmet health needs, assist with providing vital referrals and resources and impact health outcomes.

Objectives: This study aimed to assess the feasibility of implementing a SDoH screening program in an inpatient setting by screening 75% of all patients admitted to the pediatric hospital medicine (PHM) service over a 8-month period. The secondary aim was to ensure 90% of positive screens received indicated referral and/or patient navigation.

Design/Methods: This was a quality improvement initiative at a quaternary pediatric academic center in New York City. Plan-do-study-act cycles were used to implement a SDoH screen for patients admitted to the Pediatric Hospital Medicine (PHM) service. Using both electronic and paper screening workflows, volunteers, residents and nursing staff were trained on implementation and documentation of the Center for Medicaid and Medicare Innovation (CMMI) Health-Related Social Needs screening tool. The outcome measures included: 1) proportion of patients receiving indicated resources, and 2) proportion of patients connected to health navigator. Process measures included the proportion of patients screened and needs identified. Balancing measures to be tracked over time include staff satisfaction, readmission rate and length-of-stay.

Results: Over an 8-month period, 534 patients (62%) admitted to the PHM service were successfully screened for SDoH. At least one need was identified in nearly half (42%) of all patients and 98% of those with positive screening received an appropriate referral. Health navigation was offered and accepted by 63% of eligible families. Food insecurity was the most common need identified (22%), followed by housing needs (21%). Transportation, utility and safety needs were less common (12%, 5% and 6%, respectively).

Conclusions: It is feasible to implement universal screening and referral of SDoH in an inpatient setting at a large academic medical center. This study identified unmet health needs in hospitalized children not previously addressed. Future aims are to investigate the association between addressing social needs and the impact on child health outcomes.

Pediatric Patients with PTC and a Structural Incomplete Response: Effect of Distant Metastasis, Histopathology and I-131 Dose on the Need for a Second Intervention

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Objective: To determine short-term responses and long-term outcomes in pediatric patients treated with total thyroidectomy and I-131 and to define factors that affect outcomes, with focus on patients with a structural incomplete response.

Methods: Retrospective chart review of 78 patients ≤ 18 years of age at diagnosis (median age 14 years) with PTC treated with total thyroidectomy and I-131 between 2002 and 2020 in a high-volume thyroid cancer center. Histopathology, TNM staging, initial ATA risk stratification, initial I-131 dose, and need for additional interventions (second dose of I-131 or surgical intervention) were retrieved. Treatment response was determined at 6-24 months as complete (CR), indeterminate (IR), biochemical (BI), or structural incomplete (SI) response based on imaging and biochemical findings. Long-term outcomes defined as no evidence of disease (NED), recurrence, structural or biochemical persistence were determined after a median follow-up of 8 years.

Results: Among the entire cohort, 23/78 (29%) had CR, 30/78 (38%) SI, 22/78 (28%) IR, and 3/78 (3.8%) BI. There was a statistically significant difference in the lymph node status ($p=0.017$) and histopathology ($p=0.048$) between patients with complete and incomplete responses. Patients with N1 disease and diffuse sclerosing variant were more likely to have an incomplete response. All with CR continued to have NED for a median of 6.5 years after initial response. Among patients with an initial SI response ($n=30$), none achieved NED status without additional intervention, and 23/30 (77%) required a second intervention. Of these, 1/23 (4%) achieved NED, and 15/23 (65%) continued to have persistent disease. Histopathology, distant metastasis, and I-131 dose did not play a role in time to second intervention.

Discussion: Our data suggest that patients with an initial complete response after thyroidectomy and I-131 therapy have good long-term outcomes. Patients with an initial structural incomplete response often have persistent disease even after additional interventions and rarely achieve NED. This information will help providers and patients in the expectations for longitudinal outcomes following initial treatment.

Epidemiology of Platelet Transfusions in Hospitalized Children: A PHIS Database Study

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Introduction: Platelet transfusions are frequently prescribed to children. However, they are associated with significant morbidity and mortality. While multiple studies have characterized the prevalence of platelet transfusions in particular subsets of pediatric patients, few studies have described the epidemiology and complications of pediatric platelet transfusions across a wide variety of hospital settings or periods of time.

Objective: To describe the epidemiology and complications of platelet transfusions among hospitalized pediatric patients during 2010-2019.

Methods: We performed a retrospective cohort study of hospitalized children within the Pediatric Health Information System (PHIS) database. Pediatric encounters (age 0-18 years) receiving at least one platelet transfusion during hospitalization from 2010-2019 were identified. Data regarding patient demographics, admitting diagnosis, complex chronic conditions, procedures required during hospitalization, complications, and outcomes were extracted for eligible encounters.

Results: Within the PHIS database, 6,284,264 hospitalizations occurred from 2010-2019. 244,464 hospitalizations required at least one platelet transfusion, yielding a prevalence of 3.89% (95% CI: 3.87-3.91%). Transfusion prevalence did not change significantly across the decade (p -value = 0.152, range = 3.69-4.28%). Two-thirds of children receiving platelet transfusions were in their first six years of life, and the majority identified as male (55%) and white (61%). Recipients most commonly had diseases of the circulatory system (21%, 52,008/244,979), perinatal disorders (16%, 38,054/244,979), or diseases of the hematologic and/or immune system (15%, 37,466/244,979), and 91% had complex chronic conditions. Many required mechanical ventilation (44%), underwent a procedure in the operating room (53%), or were supported by extracorporeal membrane oxygenation (ECMO) (5%). When adjusted for age, need for support by ECMO, mechanical ventilation, and surgical intervention, the odds of thrombosis, infection, and mortality increased by 2% (OR 1.02, 95% CI: 1.016-1.020), 3% (OR 1.03, 95% CI: 1.028-1.033), and 7% (OR 1.07, 95% CI: 1.067-1.071) respectively with each additional transfusion.

Conclusion: The prevalence of platelet transfusions among pediatric inpatients remained consistent over the past decade. Increasing numbers of platelet transfusions are associated with increased morbidity and mortality, highlighting the need to develop more stringent transfusion guidelines in pediatric patients.

Predicting Shock in Multisystem Inflammatory Syndrome in Children: A Multicentered Analysis from the NYC Tri-State Region

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Background: Multisystem Inflammatory Syndrome in Children (MIS-C) is an uncommon but serious condition related to COVID-19. While some children may present with shock upon arrival or early in the emergency department (ED) course, others may develop shock later. Understanding the timing and factors that predict this progression would enable clinicians to assign the appropriate level of care and disposition.

Objective: We aimed to determine the clinical factors independently associated with the development of shock at ≥ 3 hours after ED arrival in children with MIS-C.

Design/Methods: We conducted a retrospective study of children 3 months to 21 years of age evaluated for MIS-C at 22 participating pediatric EDs in the New York City tri-state area from April 1 - June 30, 2020. We identified eligible patients by ICD-10 code and electronic medical review. We included patients with fever and C-reactive protein performed and defined MIS-C based on the World Health Organization criteria. Equivocal cases of MIS-C were categorized by consensus. Using a standard tool, we collected demographic, clinical, and laboratory data. Our primary outcome was MIS-C with shock occurring ≥ 3 hours after initial ED arrival to identify those who clearly did not require initial critical care. We defined shock as the presence of systolic hypotension or clinical hypoperfusion requiring a vasoactive agent or > 40 ml/kg intravenous fluid administration. We performed bivariable

and subsequent multivariable logistic regression analyses to identify factors independently associated with the outcome. For regression, we dichotomized continuous variables based on the sample median.

Results: We identified 261 patients with MIS-C, of whom 35 (13%) had shock < 3 hours of ED arrival, and excluded them from further analyses. Of the remaining 226, 85 (38%) developed shock $\geq$ 3 hours after initial ED arrival. Bivariable analysis identified 10 variables significantly associated with our outcome (Table 1). A multivariable regression found low lymphocyte count (< sample median, 16%) (adjusted odds ratio (aOR) 3.23; 95%CI: 1.47, 7.28) and low absolute platelet count (< sample median, 225 cells x 1000/uL) (aOR 3.67; 95%CI: 1.76, 7.98) were independently associated with shock $\geq$ 3 hours from ED arrival (Table 2).

Conclusion(s):

MIS-C with shock occurred in nearly half of children included in this large multicenter cohort. Low lymphocyte and platelet counts were independent factors strongly associated with the development of shock $\geq$ 3 hours from ED arrival.

EARLY CD4 T CELL IMMUNE RECONSTITUTION AFTER HCT ASSOCIATED WITH REDUCED NON-RELAPSE RELATED MORTALITY BUT NOT WITH DECREASED RELAPSE RISK

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Introduction: Early CD4+ immune reconstitution (CD4IR) after allogeneic hematopoietic cell transplant (allo-HCT) correlates with lower non-relapse mortality (NRM) and higher overall survival (OS). The role of CD4IR on relapse risk (RR) and graft-versus-host disease (GvHD) has been less clear and has not been studied in large cohorts.

Objective: We studied the impact of CD4IR on post-HCT outcomes in a large cohort of 482 pediatric and young-adult patients.

Methods: We retrospectively analyzed data from patients receiving their first allo-HCT for a malignant indication at three large academic medical centers between 2008-2019. Early CD4IR was defined as achieving >50 CD4+ T cells/ μ L at two consecutive measures within 100 days of HCT. Main outcomes of interest were RR and NRM. Fine and Gray competing risk models and Cox proportional hazard models were used.

Results: 482 patients were included; median age at HCT was 12 years (interquartile range 5.6–16 years), 198 (41.1%) were female. Indications for HCT were ALL (n=208), AML (n=179), MD S (n=41), non-Hodgkin lymphoma (n=18), MPAL (n=11) and others (n=25). The cumulative incidence of CD4IR at day 100 was 76.6% (95% confidence interval [CI] 72.8-80.3%). Younger patients (<18 yrs) had a higher incidence of CD4IR ($p < 0.001$). In multivariate analysis, achieving CD4IR was a predictor of superior OS (hazard ratio [HR] 0.49, 95% CI 0.35-0.70, $p < 0.001$), a lower risk of NRM (HR 0.26, 95% CI 0.15-0.43, $p < 0.001$) and better CRFS (HR 0.61, 95% CI 0.44-0.83, $p = 0.002$), but there was no difference in RR (HR 1.26, 95% CI 0.8-1.99, $p = 0.31$). A trend towards lower incidence of extensive cGvHD was observed in patients who reconstituted CD4+ early (HR 0.49, 95% CI 0.23-1.02, $p = 0.058$).

Conclusion: Our data confirm in a large pediatric and young adult cohort that early CD4IR is associated with a 4-fold lower NRM and hence superior survival, without any correlation with relapse. Impact of CD4IR on cGvHD needs further investigation. Other lymphocyte subsets involved in immune reconstitution should be considered in future studies to identify predictors for relapse risk.

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

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Thromboembolism (TE) is a common complication in cancer patients and the second leading cause of cancer-related deaths. TE incidence varies in different cancer types, with the highest risk in lung cancer and pancreatic ductal adenocarcinoma (PDAC), and in advanced-stage and metastatic cancers. Despite the benefits associated with thromboprophylaxis for symptomatic TE, the prevention of TE still remains an unmet clinical need due to lack of biomarkers predictive of TE risk and the bleeding risk associated with the routine use of anti-coagulants. Exosomes are small circulating extracellular vesicles that mediate cell-to-cell communication. Cancer cells and the tumor microenvironment release large numbers of exosomes into the blood circulation and have displayed a therapeutic and predictive value in systemic diseases. Integrins expressed on the surface of exosomes drive their selective organotropism and prepare distant sites for metastatic seeding by establishing favorable pre-metastatic niches. Here we show that exosomes from metastasis-bearing lungs or pre-metastatic lungs of mice with melanoma, breast, lung and pancreatic cancer induce TE in mice and express high levels of integrin beta 2 (ITGB2). Instead, exosomes from tumor cell lines, primary tumors or other metastasis-bearing organs did not show any pro-thrombotic properties. Myeloid cells including monocytes/macrophages and neutrophils infiltrating pre- and post-metastatic lungs were the main source of ITGB2+ pro-thrombotic exosomes. Blockade of ITGB2 on lung-derived exosomes, or systemically in mice, prevented exosome-induced platelet aggregation and TE, and reduced metastasis. Examination of the mechanisms of ITGB2-induced TE showed that exosomal ITGB2 interact directly or through fibrin with different binding partners on platelets, and induce their activation and aggregation. Importantly, we found that exosomal ITGB2 levels are elevated in the plasma of PDAC patients prior to TE events in comparison to PDAC patients with no history of TE, and thus might serve as prognostic biomarker of TE. Together, our results provide the first evidence of the establishment of a pro-thrombotic lung niche in different cancer types. Moreover, we identify exosomal 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.

Identifying Biomarkers of Disease in Children with Juvenile Dermatomyositis

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Introduction: Juvenile dermatomyositis (JDM) is an idiopathic inflammatory myopathy characterized by characteristic rashes, proximal muscle weakness, and heterogenous systemic involvement. While mortality has decreased with nonspecific immunosuppressive treatments, patients with JDM continue to have significant morbidity and disease pathogenesis remains poorly understood. T and B cell interactions are thought to contribute to pathogenesis and there is increasing interest in the extrafollicular compartment in the study of autoimmune diseases.

Objectives: To identify markers of disease in children with JDM, which are needed to better understand the pathogenesis and ultimately improve treatment for patients with JDM.

Methods: Flow cytometry immunophenotyping using peripheral blood mononuclear cells (PBMCs) was used to examine an initial cohort of 35 patients with JDM and 14 healthy controls (HC). A validation cohort of 30 patients with JDM and 10 HC was analyzed with an expanded flow cytometry panel. Laboratory tests and clinical measures of disease activity were recorded, including Manual Muscle Testing (MMT-8) and Childhood Myositis Assessment Scale (CMAS). Single cell RNA sequencing of PBMCs was then performed on a cohort of 24 patient with JDM and 12 HC.

Results: An expansion of extrafollicular T helper 2 memory cells (CD4+CD45RA-CXCR5-CCR6-CXCR3-) was appreciated in JDM patients compared to HC in both the initial and validation cohorts. Extrafollicular switched memory (EFSM) B cells (CD20+CD27+IgD-CXCR5-) were significantly increased in frequency and absolute numbers in JDM patients compared with HC in the initial cohort. The validation cohort similarly demonstrated a higher proportion of EFSM B cells (CD19+CD27+IgD-CXCR5lo/-) in JDM patients compared to HC. EFSM B cell frequency correlated with serum aldolase levels, and EFSM absolute cell numbers negatively correlated with MMT-8 and CMAS in the initial cohort. The initial cohort demonstrated a positive correlation between expansion of EFSM B cells and CXCR5- T helper 2 cells. Single cell RNA sequencing revealed three populations of memory B cells, conventional follicular memory B cells, DN2 cells, and the final population consistent with the EFSM B cell identified via flow cytometry.

Conclusions: These findings suggest that extrafollicular T helper 2 cells and EFSM B cells may contribute to JDM pathogenesis.

DNMT1 INHIBITORS INDUCE EXPRESSION OF TRANSPOSABLE ELEMENTS IN BURKITT LYMPHOMA CELL LINES

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Introduction: Burkitt Lymphoma (BL) is the most common type of non-Hodgkin lymphoma (NHL) in children and a subset of pediatric BL is associated with Epstein-Barr Virus (EBV) infection. While there have been advances in the immunotherapy to treat latency III EBV-infected lymphomas, most of EBV+ lymphomas, including BL, remain in a restricted latency I program with little expression of immunogenic viral genes. We previously found that latency I BL can be converted to latency III upon treatment with the DNMT1 inhibitor decitabine and that this conversion sensitizes BL to killing with EBV-directed T-cells.

Objectives: Here we sought to determine the effect of decitabine and another DNMT1 inhibitor, GSK3685032, on the expression of human endogenous retroviral (HERV) sequences. HERVs contribute up to 8% of the human genome and their expression is tightly controlled in healthy tissue. However, previous research has shown that the expression of these retroviral sequences is dysregulated in various malignancies and that translated HERV proteins can elicit robust T-cell responses as neoantigens.

Methods: To determine the impact of epigenetic therapies on EBV+ BL latency I HERV expression, we treated two latency I BL cell lines, Mutu and Kem, with decitabine or GSK3685032 and compared their transcriptomes with those of DMSO-treated cells and matched Latency III isogenic pairs (Mutu III and Kem III). With RNA-seq, we utilized a bioinformatic tool, Telescope, to analyze differential expression of locus-specific retroelements between treated and untreated cells and between latency I and latency III pairs.

Results: We show that decitabine and GSK3685032 increased the expression of 123 and 203 HERV loci, respectively. To determine the effects of treatment independent of switching latency, we compared these profiles with those of matched Latency III cells and show that decitabine induced 4 unique HERV loci and GSK3685032 induced 46 unique loci. Intriguingly, GSK3685032 induced the upregulation of ERVH-5, which has previously been shown to elicit T-cell reactivity in hematological cancers.

Conclusions: These results demonstrate that epigenetic adjuvant therapy induces expression of potentially immunogenic HERVs, which may serve as T-cell targets for immunotherapy and highlight a novel mechanism to sensitize latency I EBV+ lymphomas to immunotherapy.

Quantifying Intraventricular Drug Delivery Utilizing Programmable Ventriculoperitoneal Shunts as the Intraventricular Access Device

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INTRODUCTION: Programmable ventriculoperitoneal shunts (pVP shunts) are increasingly utilized for intraventricular chemotherapy, radioimmunotherapy, and/or cellular therapy. Shunt adjustments allow optimization of drug concentrations in the thecal space with minimization in the peritoneum.

OBJECTIVES: This report assesses the success of the pVP shunt as an access device for intraventricular therapies. Quantifying intrathecal drug delivery using scintigraphy by pVP shunt model has not been previously reported.

METHODS: We performed a single-institution, retrospective analysis on patients with CNS tumors and pVP shunts from 2003-2020, noting shunt model. pVP flow was evaluated for consideration of compartmental radioimmunotherapy (cRIT) using ^{111}In -DTPA uptake scintigraphy. Scintigraphy studies were performed at 2-4 hours and again at 24 hours post ^{111}In -DTPA injection. Images were analyzed to quantify ventricular-thecal and peritoneal drug activity by using Regions of Interest (ROI), which were manually drawn around structures exhibiting ^{111}In -DTPA uptake.

RESULTS: Twenty-two CSF flow studies were administered to 15 patients (N=15) with diagnoses including medulloblastoma, metastatic neuroblastoma, pineoblastoma, and choroid plexus carcinoma. Six different types of pVP models were noted. On qualitative analysis, 100% of the CSF flow studies demonstrated ventriculo-thecal drug activity grossly visible by imaging both at 2-4 hours and at 24 hours post injection. Also qualitatively, both at 2-4 hours and at 24 hours post injection, 27% (6 of 22) of the studies had no peritoneal uptake grossly visible by imaging alone, while 73% (16 of 22) had peritoneal uptake grossly visible on imaging. Using the quantification technique of calculating ROI on imaging both at 2-4 hours and 24 hours post injection, 73% (16 of 22) of the studies demonstrated minimal relative peritoneal uptake (<12%). 27% (6 of 22) of the studies demonstrated moderate relative peritoneal uptake (12-37%). No studies demonstrated peritoneal uptake above 37%.

CONCLUSIONS: All patients had successful drug delivery of ^{111}In -DTPA to the ventriculo-thecal space. 73% of the patients had minimal relative (<12%) peritoneal drug uptake. Though efficacy varies by shunt model, low numbers preclude conclusions regarding model superiority. CSF flow scintigraphy studies assess drug distribution of ^{111}In -DTPA, informing CSF flow for delivery of intraventricular therapy, radioimmunotherapy, and/or cellular therapy.

Timing of Tracheostomy in Critically Ill Infants and Children with Respiratory Failure: A PHIS Study

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Authors: Priyanka Mehrotra, Charlene Thomas, Linda Gerber, Ali Maresh, and Marianne Nellis

Introduction: Tracheostomy placement in children with prolonged respiratory failure has steadily increased in the past decade, yet there is no consensus for optimal timing.

Objectives: We sought (1) to describe the timing of tracheostomy and associated demographic and clinical characteristics in a large pediatric intensive care unit (ICU) cohort and (2) to compare the clinical outcomes between subgroups based on timing of tracheostomy.

Methods: This is a retrospective observational study using the Pediatric Health Information System (PHIS). PHIS was queried for all patients who underwent tracheostomy from 2010 to 2020, and patients were included if <18 years, admitted to the pediatric or neonatal ICU from 2010-2020, and with documented need for mechanical ventilation (MV) prior to tracheostomy. Patients were separated into early - ET (defined as placement at <14 days from start of MV), late - LT (placement 15-60 days from start of MV), and extended - ExT (placement >60 days from start of MV). Primary endpoints included description of demographic and clinical characteristics. Secondary endpoints included patient outcomes including length of stay (LOS), hospital cost, and mortality.

Results/Conclusions: 3557 patients underwent tracheostomy at 52 children's hospitals, of which 2245 were included in the final analysis. 46% (1043/2245) underwent ET, 40% (908/2245) underwent LT and 13% (294/2245) underwent ExT. The median (IQR) age (in months) for the total cohort was 15 (3-93) and was significantly different between the three groups with ET being the oldest ($p < 0.001$). Significant differences were observed in region, Pediatric Medical Complexity Algorithm (PMCA) category, and principle diagnosis. The majority of children in all subgroups had complex, chronic conditions. A multivariate regression analysis showed that ET was associated with lower mortality ($p < 0.001$), shorter hospital LOS ($p < 0.001$), lower hospital costs ($p < 0.001$), and decreased hospital acquired pneumonia (HAP) ($p = .001$) as compared to those children who underwent LT or ExT. In a large cohort of pediatric patients with prolonged respiratory failure who underwent tracheostomy in the US, a majority of patients had complex chronic disease. Tracheostomy within 14 days of MV was associated with improved in-hospital outcomes. After completing a multivariate regression analysis, ET was associated with decreased mortality, hospital LOS, hospital costs, and HAP when compared with LT and ExT.

Investigating maternal antibody responses to primary CMV infection during pregnancy as immune correlates of protection against placental CMV transmission in a nonhuman primate model

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Congenital cytomegalovirus (cCMV) is the most common in utero infection and causes major neurodevelopmental deficits, but there remains no licensed vaccine to prevent cCMV. Little is known about maternal immune responses that can prevent placental CMV transmission, which could guide rational design of an effective vaccine. Using the rhesus macaque (RM) model of primary RM CMV (RhCMV) infection during pregnancy, we established that pre-existing RhCMV-neutralizing IgG protected against cCMV, even in the setting of CD4⁺ T cell depletion, where vertical transmission occurs consistently in RhCMV-seronegative R MS. However, the antibody functions mediating this protection have not been fully defined. We measured IgG binding to whole virion and key glycoproteins, neutralization, antibody dependent cellular phagocytosis (ADCP), and cytotoxicity (ADCC) of RhCMV-specific antibodies and maternal plasma viral load in seronegative pregnant R MS that were infected at the end of the first trimester. 4/9 immunocompetent da MS, 6/6 CD4⁺ T cell-depleted da MS, 2/3 CD4-depleted and high RhCMV binding IgG-infused da MS, and 0/3 CD4-depleted and high RhCMV neutralizing IgG-infused da MS transmitted RhCMV to their fetuses within 4 weeks of maternal infection in all but one transmitter. Immunocompetent pregnant R MS developed both RhCMV-specific neutralizing and Fc-mediated effector functions (ADCP and ADCC) within 2-6 weeks post infection, while CD4⁺ T cell depleted da MS experienced a 1-2 week delay in the development of humoral responses. To assess the impact of maternal viremia and antibody responses on transmission risk in this group of R MS (n=21), we utilized a machine learning approach to predict transmission status from these measurements. RhCMV pentamer and gB-specific IgG responses were highly correlated, so pentamer IgG binding was used to represent both binding responses. ADCC, pentamer/gB IgG binding, and maternal viral load were the most impactful on transmission risk with 70.8±15.0% accuracy for predicting transmission status. Higher ADCC and pentamer/gB IgG binding responses led the model toward non-transmitter classification, while higher maternal viral load led to transmitter classification, suggesting the importance of polyfunctional antibody responses and control of maternal viremia in prevention of vertical transmission. A rationally-designed vaccine that elicits immune responses resulting in rapid containment of viremia could reduce vertical CMV transmission.

EFFECT OF CHELATION ON CARDIAC IRON OVERLOAD IN PATIENTS WITH TRANSFUSION-DEPENDENT THALASSEMIA

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Authors: Sujit Sheth

Background: Iron overload develops in patients with thalassemia due to increased intestinal iron absorption and high iron loads in transfused red blood cells. Chronic scheduled transfusions for patients with transfusion-dependent thalassemia are necessary for normal growth, development, and optimal cardiopulmonary health.

Excess iron deposits in the liver, heart and endocrine organs and can lead to significant morbidity and mortality. Iron chelation is necessary for prevention and treatment of iron overload. Chelation has commonly been performed using continuous infusions of deferoxamine. Long-acting oral chelation agents have recently been approved.

Objectives: Evaluate how rates of and severity of cardiac iron overload in patients with transfusion-dependent thalassemia has changed over time since the introduction of long-acting oral iron chelation therapies.

Method: We performed a single-center retrospective review of patients with transfusion-dependent thalassemia to evaluate rates of cardiac iron overload. Data was collected from patients who consented to our thalassemia registry. Data collected included timing of the start of a chronic transfusion regimen, chelation history, and cardiac iron measurements based on MRI. Overall rates and severity of cardiac iron overload were analyzed and compared over time as well as based on chelation history.

Results/Conclusion: 124 patients with transfusion-dependent thalassemia on chelation therapy in our registry had at least one cardiac MRI, with most having multiple sequential scans for cardiac iron measurements. Rates of cardiac iron overload decreased over time, with 46% of patients having at least mild iron overload ($T2^* < 20$ MS) in at least one MRI measurement between 2006 and 2010, to 25% having at least mild iron overload between 2011 and 2015, and 20% having at least mild iron overload between 2016 and 2020. Rates of severe iron overload ($T2^* < 10$ MS) decreased from 28% to 6% to 4% over these time periods. Among patients who switched from subcutaneous chelation to oral chelation, 32% saw a reduction in severity of cardiac iron overload, while 4% saw an increase in severity.

Cardiac iron overload has decreased over time. Use of long-acting oral iron chelation has contributed. Further research is needed to evaluate if rates of heart failure have decreased with improvements in iron overload.

Anti-erythrocyte antibodies in pediatric Systemic Lupus Erythematosus

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Introduction: Systemic Lupus Erythematosus (SLE) is a disease characterized by the presence of auto-antibodies, immune complex deposition, and a robust type 1 interferon signature. It is known that patients with Systemic Lupus Erythematosus (SLE) often have coombs positivity in the absence of hemolytic anemia, 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. Recent work in pediatric SLE has implicated anti-erythrocyte antibodies in disease pathogenesis via opsonization of mitochondria-containing erythrocytes leading to enhanced phagocytosis and induction of type-1 interferon by myeloid cells. Little is known about these anti-erythrocyte antibodies in pediatric SLE, including their prevalence, association with disease activity and phenotype, and antigenic target or specificity.

Objectives: The objective of this project is to confirm the presence of anti-erythrocyte antibodies in pediatric SLE patients. Further, we will characterize the target antigens and functional properties of these antibodies, as well as correlate their presence with disease activity and phenotype.

Methods: Erythrocyte membrane proteins were isolated via creation of erythrocyte "ghost" membrane protein lysate. Gel electrophoresis was used to separate different proteins. To evaluate for the presence of anti-erythrocyte antibodies in pediatric SLE patients and healthy controls, serum was applied to the erythrocyte membrane lysate and presence of anti-erythrocyte protein antibodies was analyzed using Western Blot.

Results/Conclusions: 15 of 20 pediatric SLE serum samples had strongly positive IgG bands to erythrocyte protein antigens via western blot, compared to 0 of 8 healthy pediatric controls. The SLE serum samples showed reactivity against several target antigens of different molecular weights, and patterns of reactivity varied between samples. Future work will focus on identification of the target antigens, as well as correlation of autoantibody presence with opsonization and phagocytosing capacity in vitro.

Discovering novel inflammatory pathways in patients with lupus nephritis

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Abstract: Systemic lupus erythematosus (SLE) is a clinically complex and molecularly heterogeneous 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 patients to molecularly profile the kidney of patients with different classes of LN. Additionally, we study renal progenitor cells obtained from the urine of SLE patients to gain insight into the immune profile of the patients' renal epithelial cells. RNA sequencing analysis revealed that these cells maintain an activated pro-inflammatory phenotype characterized by high NF- κ B activation and an interferon type I response in a subset of SLE patients. 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.

Hypospadias and CAKUT-linked RBFOX2 is present in developing urethra, glans, and ureter

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Introduction/Objectives: The dosage-sensitive gene, RBFOX2, regulates mRNA alternative splicing. In public databases, patients were identified with genitourinary birth defects, including hypospadias and CAKUT (hydronephrosis), associated with RBFOX2 copy number variations CNVs. Work by others posited RBFOX2-mediated splicing is integral in balancing mesenchymal-epithelial cell identity. Our RNA-sequencing analysis with penises from Rbfox2-knockout mice, exhibiting CAKUT and/or hypospadias, identified disruption of this pathway. Sequencing analysis revealed evidence of RBFOX2 interaction with sex hormone signaling.

Methods: We tested the hypothesis that the developmental origins of a subset of human hypospadias is due to RBFOX2 CNVs using whole exome sequencing. To define the cell type specific expression of RBFOX2, spatiotemporal expression was imaged over a fetal time course in mouse penises (before and after sex determination), and kidneys.

Results: Whole exome sequencing data confirmed in patients with RBFOX2 CNV and damaging SNPs, these variants were a root cause for lower tract genitourinary anomalies, as well as CAKUT. Imaging experiments in the developing penis defined RBFOX2 expression as initially confined to the peri-urethral space. In this region, the most abundant urethral RBFOX2 protein signal traveled distally starting at the base of the penis as a urethral ring of signal that halted at the junction of the penile body and glans. The glans was encased with an epithelial layer of cells that were strongly RBFOX2-positive. These cells aggregated at the site of glanular urethra formation. Most RBFOX2-positive glanular epithelial cells co-expressed androgen receptor. The preputial folds had a similar epithelial net of RBFOX2- and androgen receptor-positive cells. In the fetal kidney RBFOX2 was most abundant surrounding the ureter epithelium.

Conclusions: The coincident RBFOX2 signal localization during urethral formation and hypospadias occurrence with RBFOX2 CNV, implicate RBFOX2 as a molecular player in the double zipper mechanism of urethral formation and closure. The more peripheral co-expression of RBFOX2 and androgen receptor in glanular and preputial epithelium may be part of a RBFOX2-mediated balancing of androgen and estrogen signaling. The strong presence of RBFOX2 in the proximal ureter epithelium potentially links development of hydronephrosis with Rbfox2-loss via an imbalance between mesenchymal and epithelial cells.

The Effect of Prophylactic Use of Antifibrinolytics During Pediatric Cardiac Surgery with Cardiopulmonary Bypass on Post-Operative Bleeding and Transfusion: A Systematic Review and Meta-Analysis

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Introduction: Fibrinolytic activation and platelet dysfunction are well-documented consequences of cardiopulmonary bypass (CPB) that disrupt normal hemostasis. The efficacy of antifibrinolytics in children undergoing CPB is not as clear.

Objectives: To determine the effect of intraoperative administration of antifibrinolytics [tranexamic acid (TXA), aminocaproic acid (EACA), or aprotinin] on chest tube output and blood product transfusion after CPB.

Methods: A systematic review and meta-analysis was conducted from inception to November 15, 2021. Adult-only studies, non-human studies, and case series were excluded. This study followed the PRISMA reporting guideline.

Results/Conclusions: Seventy studies including 28,735 patients were analyzed. TXA compared to placebo resulted in a mean decrease in chest tube bleeding of 9.1 (95% CI 6.0-12.3) ml/kg, p-value <0.001, platelet requirement of 2.9 (0.1-5.8) ml/kg, p-value <0.001 and plasma requirement of 4.0 (0.6-7.2) ml/kg, p-value <0.001. Aprotinin compared to placebo resulted in a mean decrease in chest tube bleeding of 4.3 (2.4-6.2) ml/kg, p-value <0.001, platelet transfusion of 4.6 (0.6-8.6) ml/kg, p-value <0.001, and plasma transfusion of 7.7 (2.1-13.2) ml/kg, p-value <0.001. EACA compared to placebo resulted in a mean decrease in chest tube bleeding of 9.2 (2.3-21.0) ml/kg, p-value <0.001, RBC transfusion of 7.2 (95% CI 2.4-12.1) ml/kg, p-value = 0.002, and platelet transfusion of 10.7 (2.9-18.5) ml/kg, p-value <0.001. TXA showed a greater decrease in chest tube bleeding when compared to aprotinin. TXA, EACA and aprotinin are potentially effective at decreasing blood loss and blood product requirement following CPB in children. When compared to aprotinin, TXA shows a greater hemostatic effect.

Are there healthcare disparities in critically ill children admitted with severe traumatic brain injury (TBI): A Database Study

Presenting Author: Jennifer Schwam (Resident, jes9383@nyp.org)

Authors: Jennifer Schwam, Marianne Nellis, Charlene Thomas, Joy Howell, and Christine Joyce

Background: Healthcare disparities have been observed in several sub-populations of critically ill children including those with critical bronchiolitis and oncologic diagnoses. Whereas outcomes of different medical and surgical management have been described, little work has been done to explore any healthcare disparities in children who suffer from severe traumatic brain injury (TBI).

Objective: We sought to determine any differences in mortality for children with severe TBI patients based on race and/or socioeconomic status.

Design/Methods: We performed a retrospective cohort study using the Pediatric Health Information System (PHIS) database, which includes 51 children's hospitals affiliated with the Children's Hospital Association. Pediatric encounters (0 to 18 years old) requiring PICU admission and invasive mechanical ventilation for TBI from 2008-2020 were included. Race and socioeconomic status (defined by type of insurance and median household income) were predictor variables. We performed a multivariate regression to account for confounders (including age, region, year, severity level, risk of mortality, presence of chronic conditions, discharge status, and cardiac arrest). Ethnicity was not accurate in our validation cohort and not included.

Results: 30,027 children were identified. Fifty-nine percent were male with a median (IQR) age of 3 (0-11). Fifty-four percent of children were White, 23% Black, 12% other, 7% 2 or more races, 2% Asian, 1% American Indian, and <1% Pacific Islander. Sixty-one percent of children had governmental insurance and 31% commercial insurance. The median (IQR) household income was \$47,815 (35,992-63,746). The overall mortality rate was 31% (9,414/30,027). As compared to white children, Asian race and 2 or more races were independently associated with increased mortality (Odds ratio "OR" 1.24, 95% CI 1.04-1.48, p=0.016 and OR 1.82, 95% CI 1.64-2.01, p<0.001, respectively). No significant differences were seen with other races. As compared to commercial insurance, there was no significant difference in mortality in children with governmental insurance (OR 0.99, 95% CI 0.93-1.06, p=0.900). As compared to children in the lowest income bracket, children in each higher income bracket had significantly lower mortality rates (see Table below).

Conclusion: In a large cohort of children with severe TBI, differences in mortality were observed based on both race and socioeconomic status. Further studies are needed to explore causality and if established, address reasons for disparities.

REVEALING THE MOLECULAR DRIVERS OF HEPATOBLASTOMA USING PLURIPOTENT STEM CELLS

Presenting Author: Robert Schwartz (Faculty, res2025@med.cornell.edu)

Authors: Vasuretha Chandar, and Angela Frankel

Introduction: Hepatoblastoma is the most common liver neoplasm in children and accounts for approximately 1% of all childhood malignancies. Five-year survival for hepatoblastoma is 59-74%; one of the lowest survival rates for childhood cancer and is dictated largely by surgical control. Therefore, an improved understanding of the mechanisms underlying this cancer will enable the development of improved and more targeted clinical therapies. Hepatoblastoma is an embryonal tumor with distinct phenotypes and whose classification and treatment is guided largely by both histology and immunohistochemical staining. The molecular defects present in hepatoblastoma have been identified but their testing and inclusion of targeted therapies into clinical therapy have not been adopted and consequently curative treatment largely consists of surgery alone. Our central hypothesis is that the most common mutation present in hepatoblastoma tumors, CTNNB1 cause differentiation defects, increased cellular proliferation, and tumorigenesis in a cell-specific manner.

Methods: The lack of robust models of hepatoblastoma in a relevant and appropriate cell type has significantly impaired our ability to better understand the molecular underpinnings underlying this cancer and to develop improved and effective clinical therapies. We used human induced pluripotent stem cells (PSC) and PSC derived hepatoblasts and hepatocyte-like cells engineered using CrispR technology to generate mutations in β^2 -catenin, the most common gene that is mutated in hepatoblastoma.

Results: We used CrispR/Cas9 technologies to generate human PSC lines with similar mutations in CTNNB1. These PSC lines have increased activation of WNT signaling but differentiate normally into hepatoblast-like cells, at which point, these cells proliferate and have differentiation defects. CTNNB1-mutant containing PSC derived hepatoblasts and hepatocyte-like cells impact cellular differentiation, proliferation, the transcriptomic and metabolomic signature, and enable tumorigenesis in vivo.

Conclusion: In contrast to mouse models where solitary CTNNB1 mutations do not result in tumor formation and similar to what is seen in human hepatoblastoma, mutations in β^2 -catenin impact cellular differentiation, proliferation, the transcriptomic and metabolomic signature, and enable tumorigenesis in vivo. Key regulators of hepatoblastoma formation were identified. Future studies of hepatoblastoma formation will enable us to identify and target pathways regulating hepatoblastoma development and growth and will lead to the development of clinical targets for hepatoblastoma treatment.

Evaluating Burnout Among Pediatric Residents During the COVID-19 Pandemic: A Mixed Methods Study

Presenting Author: Christina Tempesta (Resident, cht9117@nyp.org)

Authors: Natasha Dilwali, Laurie Gordon, Erika Abra, and Sonia Ruparell

Background: Residents involved in caring for patients with COVID-19 are at high risk of developing negative psychological consequences such as burnout, depression, and anxiety. Compared to other physicians, residents have higher rates of burnout, fatigue, and depersonalization.

Objective: We explored the impact of the COVID-19 pandemic on resident burnout and sought program-level solutions to mitigate burnout.

Methods: We conducted focus groups and administered the Maslach Burnout Inventory (MBI) to residents in a medium-sized, academic pediatric residency program in New York City. A constant comparison analysis was used to derive themes from focus groups until we achieved thematic saturation. Burnout on the MBI was defined as a high subscale score for the emotional exhaustion domain (≥ 27), and/or the depersonalization domain (≥ 10), and/or a low score in the personal achievement domain (< 33). Scores were compared to results from residents in the same program in 2019.

Results: Five focus groups were conducted among second and third-year residents ($n=17$ of 40). The MBI was completed by 16 of these residents. Five themes were identified (Table 1). First, burnout is accentuated during a pandemic due to pervasive feelings of personal isolation. Second, residents need program support to acknowledge the extent of the disruption as well as loss of normal coping strategies. Third, program support should ensure residents feel recognized for efforts above and beyond what ordinarily would be expected. Fourth, cultivating a culture of transparency around administrative decisions is essential. Lastly, burnout can be reduced through small program changes such as redistributing resident workload. 75% of respondents met the criteria for burnout from March to June 2020 compared to 61% in 2019. Depersonalization remained the highest subscale score (Table 2, 61% pre-pandemic, 56% during the pandemic). Percentage of residents with emotional exhaustion increased (Table 2, 10% pre-pandemic, 50% during the pandemic).

Conclusions: Our study demonstrates pandemic burnout is unique and requires different mitigation and support strategies at the program level. By understanding the specific factors that contribute to burnout, early interventions can be implemented, which may mitigate the rising rates of depersonalization and emotional exhaustion seen throughout the pandemic.

Flattening of circadian glucocorticoid oscillations drives acute hyperinsulinemia and adipocyte hypertrophy

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Disruption of circadian glucocorticoid oscillations in Cushing's disease and chronic stress results in obesity and adipocyte hypertrophy, which is believed to be a main source of the harmful effects of obesity. Here we recapitulate stress due to jet lag or work-life imbalances by flattening glucocorticoid oscillations in mice. Within 3 days, mice achieve a metabolic state with persistently high insulin, but surprisingly low glucose and fatty acids in the bloodstream, that precedes a more than two-fold increase in brown and white adipose tissue mass within three weeks. Transcriptomic and Cd36-knockout mouse analysis show that hyperinsulinemia-mediated de novo fatty acid synthesis and Cd36-mediated fatty acid uptake drive fat mass increases. Intriguingly, this mechanism by which glucocorticoid flattening causes acute hyperinsulinemia and adipocyte hypertrophy is unexpectedly beneficial in preventing high levels of circulating fatty acids and glucose for weeks, thus serving as a protective response to preserve metabolic health during chronic stress.

Longitudinal Assessment of Leydig Cell Function in Male Survivors of Childhood Cancer

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Introduction & Objectives: As the number and longevity of childhood cancer survivors increases, it is becoming more important to assess long term treatment-associated health risks. The objective of this study was to longitudinally examine the incidence, associated factors, and risk factors for Leydig cell dysfunction (LCD) and Leydig cell failure (LCF) in men who underwent treatment for pediatric cancers.

Patients & Methods: We performed a retrospective chart review of available data from male patients treated during childhood for various cancers at MS KCC. The main outcome variables of interest were serum testosterone and LH levels during childhood and adulthood. We examined risk factors for testosterone insufficiency including history of stem cell transplant, treatment with total body irradiation (TBI), and cyclophosphamide equivalent dose (CED).

Results: Out of 118 eligible male subjects, 7.6% had Leydig cell failure (low serum testosterone level), and 14.4% had Leydig cell dysfunction (elevated LH level with normal testosterone level). Subjects who were sufficient in testosterone in adulthood (N = 105) remained sufficient for a mean of 11.1 years after treatment. We found significant associations between LCF and treatment with TBI ($p < 0.003$), and between LCF in adulthood and testosterone insufficiency in childhood ($p < 0.001$). We did not find an association between LCF and CED greater than or less than 20 g/m² ($p = 0.2$).

Conclusions: Incidences of Leydig cell failure and Leydig cell dysfunction are low in male survivors of childhood cancer. Longitudinally, there is an association between childhood testosterone insufficiency and Leydig cell failure in adulthood. Despite low incidence, in those who are affected with LCF, TBI is a risk factor. Alkylating agents and transplant alone do not appear to be risk factors for Leydig cell failure.

Exercise-induced exosomes provide a therapeutic potential for breast cancer prevention

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Epidemiological evidence suggests a high correlation between physical activity and cancer protection and survival. However, the underlying cellular mechanisms remain elusive. Exosomes are small vesicles released by all cell types and efficient messengers in tissue crosstalk. We hypothesize that exosomes mediate tissue crosstalk during exercise and may be responsible for the benefits of exercise in cancer prevention and survival. Here we propose to: 1) characterize the source and unique cargo of exosomes mediating cancer prevention; 2) determine the mechanisms by which these exosomes prevent cancer growth and metastasis; and 3) identify the exosome cargo from patients undergoing controlled exercise programs responsible for the beneficial effects of exercise, that can represent the basis of novel preventive or therapeutic strategies.

Breadth and Potency of Humoral Immunity Elicited by Multivalent HCMV glycoprotein B mRNA vaccines

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Human cytomegalovirus (HCMV) remains the most common cause of congenital infections and complications in immunocompromised patients. The most successful HCMV vaccine to-date was a HCMV glycoprotein B (gB) subunit vaccine adjuvanted with MF59 that achieved 50% efficacy against the acquisition of primary HCMV infection in clinical trials. A previous study found that gB/MF59 vaccinees were less frequently infected with HCMV strains encoding gB genotypes most like the autologous vaccine strains (1, 2, and 4) than strains encoding heterologous gB genotypes (3 and 5). This study suggests the gB/MF59 vaccine elicited strain-specific protective immunity, possibly leading to the limited efficacy. We hypothesized that a broader protection against multiple HCMV gB genotypes may be required to increase the vaccine efficacy.

We immunized 18 female rabbits with monovalent (gB1), bivalent (gB1+gB3), or pentavalent (gB1+gB2+gB3+gB4+gB5) gB mRNA vaccines to test whether a nucleoside-modified mRNA-lipid nanoparticle vaccine encoding multiple HCMV gB genotypes could broaden anti-CMV immunity. Rabbit plasma from the multivalent vaccine groups did not elicit a higher IgG response to gB ectodomain genotypes 3 and 5 compared to that of monovalent vaccine group. Since IgG binding to cell-associated gB was found as a correlate to vaccine efficacy in gB/MF59 clinical trial, this response against 5 genotypes was measured yet no significant differences were observed among three groups. The multivalent vaccine groups elicited a slightly higher plasma neutralization capability against three HCMV strains, while no significant differences were observed in antibody-dependent cellular phagocytosis response among three groups. Our data suggests that the multivalent gB mRNA antigens does not significantly increase the breadth of anti-CMV antibody responses, therefor inclusion of mosaic or additional glycoproteins in vaccines may be more beneficial to enhance breadth and efficacy.

The Magnitude and Breadth of SARS-CoV-2 Vaccine-Elicited IgG Response in Breastmilk

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Authors: Mackensie Gross, Savannah Herbek, Sallie Permar, and Yawei Jenny Yang

Introduction: The effect of SARS-CoV-2 mRNA vaccines on breast milk antibody remains understudied. We conducted a longitudinal study (n=13 participants) to investigate the breast milk antibody kinetics during and after immunization with a SARS-CoV-2 mRNA vaccine.

Objectives: To characterize Spike specific IgG in the breastmilk of women immunized with a SARS-CoV-2 mRNA vaccine.

Methods: Breastmilk samples were collected weekly starting at the first dose of vaccine until two weeks after the second dose, and monthly for six months afterwards. ELISA was used to calculate the concentration of SARS-CoV-2 Spike-specific IgG. A Binding Antibody Multiplex Assay (BAMA) panel of 11 variant spike proteins, and nucleoprotein, was used to assess IgG binding breadth. A breadth score was calculated to characterize binding to multiple variants.

Results: All participants tested to date (n=3) had detectable spike-specific IgG in their milk, which peaked two weeks after the second dose of vaccine (range: 20-45 $\mu\text{g/mL}$). Spike-specific IgG remained detectable at three months post dose 1 yet waned between 55-60% from peak levels (range: 8-20 $\mu\text{g/mL}$). The IgG Spike-binding breadth scores calculated from one participant were 29.2 after one dose, 80.3 after two doses, and waned to 51.1 at three months post dose 1. At peak titers, IgG binding to Omicron variant spike was only 12% lower than binding to D614G spike protein, however, by three months after the first dose of vaccine, IgG binding to Omicron waned to below the negative cutoff.

Conclusions: Postpartum vaccination induces IgG in breastmilk capable of binding to multiple SARS-CoV-2 spike variants. IgG is detected at least 3 months post vaccination but binding breadth wanes at varying rates. The impact of infant exposure to these IgG remains unknown.