Center for Childhood Infections & Vaccines

9th Annual Symposium

November 6th, 2025

9:00 AM – 4:00 PM

Health Sciences Research Building (HSRB-I/II)

Rollins Auditorium and Atrium

sanofi

Pfizer

EMORY UNIVERSITY

Children's Healthcare of Atlanta

MOREHOUSE SCHOOL OF MEDICINE

Georgia Institute of Technology

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Welcome

Dear Colleagues,

Welcome to the 9th Annual Center for Childhood Infections and Vaccines (CCIV) Symposium! We are thrilled to have you join us for what promises to be an informative and engaging event.

This year's symposium features 38 abstracts showcasing the outstanding work of our faculty, staff, trainees, and students. Our agenda is packed with presentations highlighting cutting-edge infectious disease and vaccine research taking place at Emory University, Children's Healthcare of Atlanta, and our partner institutions.

This year's Symposium will feature two outstanding keynote speakers:

Paul Offit, MD
Director of the Vaccine Education Center and Professor of Pediatrics in the Division of Infectious Diseases at the Children's Hospital of Philadelphia.
Dr. Offit is one of the world's foremost authorities on vaccines, immunology, and infectious diseases. He co-invented the rotavirus vaccine *RotaTeq*, has authored 11 acclaimed books, and serves as a trusted national voice on vaccine science and policy.

Stephen W. Patrick, MD, MPH, MS, FAAP
Chair and O. Wayne Rollins Distinguished Professor of the Department of Health Policy and Management at Emory's Rollins School of Public Health.
Dr. Patrick is a nationally recognized leader in maternal and child health policy. His NIH-funded work has transformed care for families affected by substance use disorders and informed major public health initiatives at the White House, NIH, and CDC.

In addition, we are pleased to welcome our internal guest speakers, Dr. Jens Wrammert and Dr. Kristy Rostad, who will share their expertise and contributions to the field.

Thank you for taking the time to attend the symposium. We look forward to a day of stimulating discussions and collaboration.

Sincerely,

Brian Zanoni, MD
Symposium Co-Chair
Associate Professor

Rabindra Tirouvanziam, PhD
Symposium Co-Chair
Associate Professor

Mehul Suthar, PhD
Director of CCIV
Associate Professor

Our Keynote Speakers

Paul A. Offit MD
Director of the Vaccine Education Center
Children's Hospital of Philadelphia

Dr. Paul A. Offit is a leading authority in vaccinology, pediatric infectious diseases, virology, and immunology. He serves as Director of the Vaccine Education Center at the Children's Hospital of Philadelphia and is the Maurice R. Hilleman Professor of Vaccinology at the University of Pennsylvania's Perelman School of Medicine. A former member of the CDC's Advisory Committee on Immunization Practices and a current member of the FDA's vaccine advisory committee, he has shaped national vaccine policy and public understanding of immunization. Dr. Offit has published more than 160 scientific papers and co-invented the rotavirus vaccine RotaTeq, which is recommended worldwide for infants and has saved countless lives. His scientific impact has earned him numerous prestigious awards and recognition from major medical, academic, and global health organizations.

Beyond his scientific achievements, Dr. Offit is one of the most influential public voices on vaccines, science communication, and medical ethics. He has written 11 acclaimed books that challenge misinformation, defend evidence-based medicine, and explore the history of vaccine development and medical breakthroughs. His works, including *Autism's False Prophets*, *Deadly Choices*, and *You Bet Your Life*, have become essential reading for those seeking clarity on vaccines and public health. Through his research, inventions, policy leadership, and public education, Dr. Offit has played a defining role in advancing vaccine science and strengthening public trust in modern medicine.

Stephen Patrick, MD, MPH, MS, FAAP
O. Wayne Rollins Distinguished Professor and Chair
Department of Health Policy and Management
Rollins School of Public Health

Stephen W. Patrick, MD, MPH, MS, FAAP, is Chair and O. Wayne Rollins Distinguished Professor of the Department of Health Policy and Management at Emory University's Rollins School of Public Health, Co-Director of the Emory Health Services Research Center, a practicing neonatologist at Children's Hospital of Atlanta, and an Adjunct Physician Policy Researcher at RAND Corporation. His NIH-funded research focuses on improving outcomes for pregnant women with opioid use disorder and their infants, as well as families involved in the U.S. child welfare system. He previously served as Senior Policy Advisor to the White House Office of National Drug Control Policy, where he led the development of a national interagency action plan to improve outcomes for families affected by substance use and contributed to both the White House Conference on Hunger, Nutrition, and Health and the White House Blueprint for Addressing the Maternal Health Crisis. He also led Firefly, a multidisciplinary treatment program for pregnant women with opioid use disorder and their infants in Nashville, Tennessee, supported by the Centers for Medicare and Medicaid Innovation. Dr. Patrick's work includes policy analysis and public polling to inform child health policy at the state and national levels, with a focus on translating research into action. He currently serves on the NIH Organization and Delivery of Health Services Study Section, the American Academy of Pediatrics Committee on Federal Government Affairs, and

the National Academy of Medicine's Board on Children, Youth, and Families, and has served as a Guest Researcher at the CDC and a voting member on FDA advisory boards focused on pediatric opioid use. He has authored over 140 peer-reviewed publications in leading journals including The New England Journal of Medicine, JAMA, Pediatrics, and Health Affairs, and his work has been featured in PBS NewsHour, BBC, The New York Times, USA Today, and The Washington Post. His honors include the AMA Foundation Excellence in Medicine Leadership Award, the Nemours Child Health Services Research Award, the Society for Pediatric Research Young Investigator Award, and the Gale and Ira Drukier Prize in Children's Health Research. Dr. Patrick earned his degrees from the University of Florida, Florida State University College of Medicine, and Harvard School of Public Health, and completed his training in pediatrics, neonatology, and health services research as a Robert Wood Johnson Foundation Clinical Scholar at the University of Michigan.

Our Internal Guest Speakers

Jens Wrammert, PhD
Associate Professor, Emory Vaccine Center
Associate Professor, Department Pediatrics,
Division of Infectious Diseases
Emory University School of Medicine

Christina "Kristy" Rostad, MD
Associate Professor, Department Pediatrics,
Division of Infectious Diseases
Emory University School of Medicine

Networking Lunch Hosts

This year's CCIV Annual Symposium will feature a Networking Lunch in the 1st Floor Atrium of the Health Sciences Research Building (HSRB). During this special session, attendees will have the opportunity to connect directly with eight CCIV faculty members: Mehul Suthar, PhD, Rabindra Tirouvanziam, PhD, Ann Chahroudi, MD, PhD, Erin Scherer, PhD, Jens Wrammert, PhD, Vineet Menachery, PhD, Christina "Kristy" Rostad, MD, and Christopher Neufeldt, PhD. Each faculty member will host a designated table where students, trainees, and other researchers can join for informal discussions about research, career development, and collaboration opportunities. Seating will be available on a first-come, first-served basis, so participants are encouraged to arrive early to engage with their preferred faculty mentor. See below for more information about each faculty member:

Dr. Mehul Suthar is an Associate Professor of Pediatrics and Microbiology & Immunology at Emory University and serves as Director of the Center for Childhood Infections and Vaccines (CCIV). His research focuses on understanding how the immune system responds to emerging viral infections, such as Zika virus and SARS-CoV-2, and how these responses shape protection or disease severity. The Suthar Lab employs systems immunology, virology, and translational approaches to uncover immune mechanisms that inform vaccine and therapeutic design. Dr. Suthar's leadership at CCIV has been instrumental in expanding cross-disciplinary research and fostering collaborations that strengthen Emory's role in pediatric infectious disease and vaccine innovation.

Dr. Rabindra Tirouvanziam is an Associate Professor of Pediatrics at Emory University and a leading investigator in pulmonary immunology. His research investigates the immune mechanisms underlying chronic lung inflammation, particularly in cystic fibrosis and other respiratory diseases. By integrating clinical samples with cellular and molecular analyses, his lab seeks to define how immune dysregulation contributes to disease progression and to identify new therapeutic strategies that restore immune balance and improve patient outcomes.

Dr. Ann Chahroudi is a Professor of Pediatrics and Director of the Division of Pediatric Infectious Diseases at Emory University School of Medicine. Her research focuses on understanding HIV persistence and developing strategies to achieve remission in children. A leader in pediatric infectious disease research, Dr. Chahroudi was recently appointed to the NIH Office of AIDS Research Advisory Council and named Strategy and Operations Officer for the Society for Pediatric Research (SPR) for the 2025–2028 term, reflecting her national leadership and commitment to advancing child health through research and collaboration.

Dr. Erin Scherer is an Assistant Professor in the Division of Infectious Diseases in the Department of Medicine at Emory University School of Medicine. She also serves as Director of the Hope Clinic Vaccine and Treatment Evaluation Unit (VTEU) Research Laboratory and is a full member of the Microbiology and Molecular Genetics and Immunology and Molecular Pathogenesis graduate programs in Emory's Laney Graduate School. Dr. Scherer's research focuses on human B cell and antibody responses to vaccination

and infection, with the goal of developing better vaccines for viral pathogens such as influenza, HPV, and SARS-CoV-2. Her career spans extensive training at The Scripps Research Institute, University of Oxford, University of Washington, Fred Hutchinson Cancer Center, and the CDC—combining academic rigor, translational vaccine science, and leadership in immunology and infectious disease research.

Dr. Jens Wrammert is an Associate Professor in the Department of Pediatrics and a member of the Emory Vaccine Center. His research focuses on defining the human antibody response to viral infections and vaccination, with emphasis on influenza, dengue, and SARS-CoV-2. Dr. Wrammert's pioneering studies have advanced understanding of long-lived plasma cells and memory B cells, providing critical insights that inform next-generation vaccine and therapeutic antibody development.

Dr. Vineet Menachery is an Associate Professor in the Department of Pediatrics at Emory University and a renowned virologist specializing in coronavirus pathogenesis. Before joining Emory and Children's Healthcare of Atlanta, he led a research group at the Galveston National Laboratory at the University of Texas Medical Branch (UTMB). His research program focuses on two interconnected areas: the emergence and infection mechanisms of novel coronaviruses (CoV) and the impact of host factors and co-morbidities on CoV disease outcomes. A leading figure in virology, Dr. Menachery has co-authored more than 100 scientific papers and received multiple national and international awards. He currently serves as Principal Investigator for one of the NIH Antiviral Drug Discovery (AViDD) Centers and as an Investigator for the Burroughs Wellcome Fund Pathogenesis Program, advancing innovative antiviral research that bridges basic science and clinical relevance.

Dr. Christina "Kristy" Rostad is an Associate Professor of Pediatrics at Emory University and an investigator with the Emory Vaccine Center and Children's Healthcare of Atlanta. Her research focuses on vaccine-induced immune responses in infants and children, with an emphasis on respiratory pathogens such as RSV, SARS-CoV-2, and influenza. Dr. Rostad's translational work bridges laboratory science and clinical vaccine trials, aiming to improve vaccine safety, immunogenicity, and accessibility for pediatric populations worldwide.

Dr. Christopher Neufeldt is an Assistant Professor in the Department of Microbiology & Immunology at Emory University School of Medicine. He earned his BSc in Immunology and PhD in Cell Biology from the University of Alberta, where he investigated how hepatitis C virus infection alters host nuclear-transport pathways. He then completed postdoctoral training in Molecular Virology at the University of Heidelberg in Germany, focusing on how flaviviruses interact with host membrane proteins. Since joining Emory's faculty in 2021, Dr. Neufeldt's laboratory uses advanced imaging techniques alongside molecular virology and cell biology tools to explore how positive-strand RNA viruses manipulate ER membrane systems and vesicle-trafficking pathways to facilitate infection and evade immune detection. Their goal is to identify conserved host pathways that can serve as broad-spectrum antiviral targets.

Symposium Schedule

Time/Room	Presentation
8:00 – 8:45 AM	**Registration and breakfast**
Rollins Café	
9:00–9:15 AM	**Welcome and opening remarks**
Rollins Auditorium	**Mehul Suthar, PhD** Director of CCIV Associate Professor
9:15–9:45 AM	**Internal Speaker 1**
Rollins Auditorium	**Jens Wrammert, PhD** Associate Professor, Emory Vaccine Center Associate Professor, Department Pediatrics, Division of Infectious Diseases Emory University School of Medicine
10:00–11:00 AM	**Keynote I**
Rollins Auditorium	**Paul A. Offit MD** Director of the Vaccine Education Center Children's Hospital of Philadelphia
11:00–11:30 AM	**Rapid-fire Block A (5 speakers)**
Rollins Auditorium	***Shane Conyers, MPH*** *"Identifying the Optimal Schedule for a Multi-Pathogen Combination Vaccine for Diarrheal Disease"* ***Daniel Kim*** *"The TLR 7/8 Agonist, INI-4001, Induces Immune Activation and Slows Viral Load Decline in SIV-Infected Infant Rhesus Macaques"* ***William Gansereit*** *"Parental Polio Vaccine Attitude, Disease Knowledge, and Characteristics"* ***Anuradha Rao, PhD*** *"Detection of highly pathogenic avian influenza A(H5N1) in contrived nasal swab specimens using commercial molecular influenza A tests"* ***Georgios Dangas*** *Epigenetic Silencing of HBV cccDNA: Role of Histone H1-0 in Host Restriction and Viral Evasion*

11:30–12:30 PM Networking Lunch

HSRB2 Atrium

1:00–1:45 PM Keynote II

Rollins Auditorium

Stephen Patrick, MD, MPH, MS, FAAP
O. Wayne Rollins Distinguished Professor and Chair
Department of Health Policy and Management
Rollins School of Public Health

1:45–2:15 PM Internal Speaker 2:

HSRB2 Atrium

Christina "Kristy" Rostad, MD
Associate Professor, Department Pediatrics,
Division of Infectious Diseases
Emory University School of Medicine

2:15–2:45 PM Rapid-fire Block B (5 speakers)

Rollins Auditorium

Devyani Joshi
"Infants' Humoral Immune Responses to Primary Influenza Vaccination and Infection"

Mahfuza Akter
"Community-Based Household Surface Sampling: A Citizen Science Approach for Recovery of Multidrug-Resistant Enterobacterales from Household Environments"

Barrett Breeze
"Household and Environmental Factors Associated with Community Spread of Multi-Drug Resistant Enterobacterales"

Christelle Radi
"Ultrasound Imaging of Draining Lymph Nodes: A Surrogate Marker for Vaccine-Induced Immune Activation"

Katherine Shen
"Community Reservoirs of Antibiotic Resistance: Enterobacterales in Green Algae from Chicago Waterways"

2:45–3:00 PM Sponsor Remarks

Rollins Auditorium Pfizer

3:00 – 3:45 PM	Poster Session

Atrium

1. Buchanan
Pediatric Vaccine Hesitancy and Families Seeking Care in Urgent Care Centers

2. Xu
Antibody-dependent Zika virus infection of Human Placental macrophages occur predominantly through FcγRIA

3. Zhou
SARS-CoV-2 EndoU-ribonuclease regulates RNA recombination and impacts viral fitness

4. Fan
Design and Synthesis of Novel Dimeric Molecules Targeting HBV Capsid Assembly

5. Sankaranarayanan
Developing a Mouse Model to Assess mRNA Vaccine-induced Enhanced Respiratory Syncytial Disease

6. Karver
Discovery and Characterization of IFI44 as a Key Hepatocyte ISG Restricting Hepatitis B Virus Infection

7. Bellman
Clinical predictors of fatality in pediatric Rocky Mountain spotted fever cases in Sonora, Mexico 2004-2024

8.Torres Rivera
Imaging HIV-1 Restriction by MX2

9. Morgan
The furin cleavage sequence is not required for SARS-CoV-2 transmission

10. Bowers
Multi-pathogen Screening of Lesion Swab Specimens Submitted for Clinical Testing at a National Reference Laboratory

11. Cortez
Endosomal fusion of SARS-CoV-2 mediated by TMPRSS2-cleaved Spike glycoprotein

12. Verma
Multifaceted Mechanism of Inhibition of Enveloped Virus Fusion by Interferon-Induced Transmembrane Proteins

13. Biswas
Breaking the Chain: Multi-Country Insights on Adolescent Social Mixing to Guide TB Prevention Strategies

14. Carr
Modulation of Purinergic Signaling by Streptococcal Nucleotidases

15. **Shooter**
Production of rhinovirus C2-specific monoclonal antibodies using hybridoma technology

16. Moore
Methods for Estimating VE Using Routine School Testing Data with Differential Testing Behavior

17.Cockerham
CD8+ T-Cell Activation Is Associated with Partial Viremia Control after Antiretroviral Therapy Cessation in SIV-Infected Infant Macaques

18. McFadden
Modifying PF74 Improves Anti-HIV-1 Activity Against the Resistance-associated Capsid Mutation N74D

19. Kar
SARS-CoV-2 Priming Exacerbates Influenza Severity and Mortality

20. Malakar
Modeling West Nile virus infection and host response in human brain organoids

21. Weimer
From Transmission to Taxonomy: Delineating Triatoma sp. nov., a Novel Species of Chagas Disease Vector from Northern Belize

22. Sabino
Impact of Freeze-Thaw Cycles on Detection of Respiratory Syncytial Virus (RSV) Antigens and RNA When Assessing Novel Multiplex Diagnostic Assays

23. Francois
Assessing Opt-Out HIV Testing in Pediatric Emergency Departments After Two Years of Implementation

24. Komal
A phase 1 study of inactivated rotavirus vaccine CDC-9 in healthy adults

25. Zaki Pour
Experimental CD8 cell depletion induces viral reactivation in ART-suppressed SIV-infected rhesus macaque infants

26. Leach
Antibodies produced after infection with WNV-1 have reduced neutralizing ability against WNV-2

27. Edara
Anti-SIV Env RhmAbs and venetoclax during analytical therapeutic interruption as a HIV-1 cure strategy in adult rhesus macaques

28. Ferrell
Variation among DENV1 major lineages affects neutralization by sera from naturally infected and vaccinated individuals

3:45 – 4:00 PM	Awards and Closing
Atrium	Best Poster and Best Talk Award

4:00–4:30 PM	Light Refreshments and Networking
Rollins Cafe	

Identifying the Optimal Schedule for a Multi-Pathogen Combination Vaccine for Diarrheal Disease

Authors: Shane A. Conyers, Aniruddha Deshpande, Avnika Amin, Ritesh Sivakumar, Elizabeth Rogawski McQuade, Ben Lopman

Presenting Author: Shane A. Conyers, MPH

Background: Diarrheal disease causes approximately 390 million cases and 340,000 deaths annually, with rotavirus as the only vaccine-preventable etiology. A combination vaccine targeting multiple enteric pathogens could enhance protection while reducing injections, clinic visits, and costs, potentially improving adherence. However, optimizing the vaccination schedule is complex, as each pathogen differs in transmissibility, immunity, and disease age distribution. Although the highest-risk period occurs early in life, vaccination during this time risks attenuation of vaccine efficacy (VE) due to factors that hinder vaccine take in early childhood. A practical combination vaccine must not only account for these factors but also integrate into existing immunization schedules while maximizing disease reduction.

Methods: We developed a dynamic, age-structured compartmental model. Parameters included age-specific mortality rates from the Global Burden of Disease study and immunity/transmissibility estimates from the eight MAL-ED study sites. We modeled two-dose viral (rotavirus, norovirus, adenovirus 40/41) and bacterial (Shigella, ETEC) combination vaccines. Simulations evaluated five schedules (birth/6 weeks, 6/10 weeks, 10/14 weeks, 14 weeks/9 months, 9/60 months) under scenarios with and without attenuation of VE in early childhood, each at 50%, 70%, and 90% coverage. Outcomes included annual cases, deaths, and percent reductions, with medians and 95% uncertainty intervals (UIs). Sensitivity analyses examined the influence of key parameters.

Results: Assuming uniform VE, earlier schedules were most optimal: 0/6 weeks reduced cases by 34% (95% UI: 15–69%) and deaths by 56% (95% UI: 41–74%); 6/10 weeks reduced cases by 35% (95% UI: 15–70%) and deaths by 55% (95% UI: 39–74%). Assuming attenuated VE, the 6/10-week schedule was optimal in reducing both cases and deaths, resulting in reductions of 21% (95% UI: 7 to 49%) and 42% (95% UI: 29 to 58%), respectively. Rate parameters were strong predictors in cases and deaths averted across simulations.

Conclusion: All schedules reduced annual cases and deaths in most scenarios. Under uniform VE, the birth/6-week schedule most reduced deaths; otherwise, the 6/10-week schedule consistently performed best. This study underscores the importance of vaccine schedule timing and age-related variation in vaccine efficacy for programs aimed at reducing enteric burden in young children.

The TLR 7/8 Agonist, INI-4001, Induces Immune Activation and Slows Viral Load Decline in SIV-Infected Infant Rhesus Macaques

Authors KIM, DANIEL; King, Alexis; Farinre, Omotayo; Endrias, Kedan; Davis, Kaleaha; Singh, Vidisha; Whang, Patrick; Wood, Jennifer; Ehnert, Stephanie; Liang, Shan; Miller, Shannon; and Chahroudi, Ann

Presenting Author: Daniel Kim, BS

Background: Infants who acquire HIV through vertical transmission require lifelong ART due to early viral reservoir formation. Pediatric cure strategies aimed at limiting reservoir establishment through the enhancement of immune responses are being evaluated. Here, we assessed the ability of a novel liposomal nanoparticle delivering a TLR 7/8 agonist, INI-4001, to stimulate immune cells in SIV-infected infant rhesus macaques (RMs).

Methods: Six infant RMs were I.V. infected with SIVmac251, and ART was initiated one week post-infection. Four RMs received four weekly doses of INI-4001 (0.05 mg/kg), and two served as ART-only controls. Blood was collected to monitor plasma SIV-RNA by rtPCR, pro-inflammatory cytokines by multiplex assay, and immune cell activation by flow cytometry.

Results: Plasma SIV-RNA peaked at 107-108 copies/ml one week post-infection. The median viral load decline rate (log copies/ml/day) post-ART was slower in INI-4001-treated RMs compared to controls: 0.117 and 0.234, respectively, from 0-14 days and 0.079 and 0.083, respectively, from 14-42 days. Transient increases in MIP-1a, IL-6, IFNa, TNFa, and IFNg occurred 2-6 hrs post INI-4001 administration, returning to baseline levels by 24 hrs. Elevated CD169 expression on classical and intermediate monocyte subsets was found 48 hrs after the first two doses. Increased CD69 expression on CD8+ T-cells was observed 48 hrs after the first dose only.

Conclusions: INI-4001 treatment induced pro-inflammatory cytokine expression, activation of innate and adaptive immune cells, and slower initial viral load decline post-ART. Future studies will evaluate the impact of immune stimulation and latency reversal in conjunction with early ART and bNAb therapy on reservoir formation.

Parental Polio Vaccine Attitude, Disease Knowledge, and Characteristics

Authors: GANSEREIT, WILLIAM; Saint-Victor, Diane; Gethers, Casiel Tey; Radcliff, Eunice; Smith, Joy; and Shane, Andi

Presenting Author: William Gansereit, BS

Background: Parental knowledge and attitudes towards vaccination impact vaccine uptake, which is essential in maintaining population immunity. We assessed the psychometric validity of a questionnaire assessing parental knowledge, attitude, and practices regarding polio immunization status.

Methods: A questionnaire assessing polio disease knowledge and attitudes was designed and administered to parents of children receiving care at a hospital-based metropolitan primary care pediatric clinic. Psychometric analysis using RStudio (Version 2025.05) was performed to validate domains of disease knowledge and vaccine attitudes, including exploratory factor analysis (EFA) to identify underlying constructs and group questions by construct for cumulative scoring. Relationships between demographic characteristics, questionnaire constructs, and vaccination status were evaluated using mean comparison tests. Delayed polio immunization status was defined as <1 dose by 2 months of age, <2 doses by 4 months, <3 doses by 18 months, and <4 doses by 6 years.

Results: EFA supported two underlying construct groups (disease knowledge and vaccine attitude) with a strong model fit (RMSEA < 0.001; TLI=1.009). Parents with lower educational attainment ($F = 3.095$, $p = 0.018$) and non-full-time employment ($F = 4.034$, $p = 0.004$) had significantly lower knowledge scores. Number of discreet responses ("I don't know" or "Prefer not to say") was significantly associated with the responder's employment status ($F = 2.492$, $p = 0.044$). On-time polio immunization was associated with more discreet responses ($p < 0.001$) and from respondents with older children ($p < 0.001$) compared to delayed polio-immunization. Discreet responses were also higher among parents who accepted vaccination at the time of recruitment compared to those who refused ($p = 0.030$). Higher composite vaccine attitude scores (indicating negative attitude) were strongly associated with vaccine refusal ($p = 0.016$), supporting the convergent validity of this instrument in distinguishing polio vaccine acceptance behaviors.

Conclusions: Both explicit concerns about vaccine safety and efficacy, as well as patterns of ambiguous responding, reflected caregiver hesitancy, attitude, and knowledge gaps, which led to delayed or complete avoidance of polio vaccination. Identifying and addressing the needs of parents with explicit concerns and ambiguous responses is critical in improving polio vaccine uptake and maintaining polio eradication.

Detection of highly pathogenic avian influenza A(H5N1) in contrived nasal swab specimens using commercial molecular influenza A tests

Authors: Bassit, Leda; Damhorst, Gregory L.; Bowers, Heather B.; Sabino, Courtney; Williams, Evelyn Kendall; Sullivan, Julie; Kennedy, Emily B.; Khouri, Jacob; Miller, Pamela; Lai, Eric; Schinazi, Raymond F.; Rogers, Beverly B.; Lam, Wilbur; Pollock, Nira R.; and RAO, ANURADHA

Presenting Author: Anuradha Rao, PhD

Background: At least 70 human cases of highly pathogenic avian influenza (HPAI) A H5N1 (i.e., "bird flu") have been reported in the U.S. since 2024, primarily among individuals with exposure to dairy cattle or poultry. These cases, along with the rapid transmission of bird flu among U.S. wild bird, poultry, and cattle populations, warrant testing of available influenza A diagnostics for their ability to detect 2024 H5N1 strains. We previously tested 12 lateral flow assays (LFAs) and 5 point-of-care (POC) nucleic acid amplification tests (NAATs) with influenza A(H5N1) clade 2.3.4.4b (https://doi.org/10.1101/2025.04.15.25325613) as part of a pandemic preparedness assessment. Here we expand the analysis by testing six additional FDA-cleared small footprint, sample-to-answer NAATs. The tests are anonymized pending authorization from each company.

Methods: We performed testing with specimens contrived with heat-inactivated 2024 H5N1 clade 2.3.4.4b genotype B3.13 (bovine, BEI, NR-59872) by spiking negative nasal swab matrix with a known quantity of virus and producing a dilution panel. Fifty microliters of contrived specimen was added directly to a swab and the swab added to 3.0 mL UTM; each dilution was tested in triplicate for each of six assays according to respective instructions for use. The lowest dilution producing 3/3 positive results was recorded as the detection limit. The dilution panel was also tested with the CDC H5 genotyping assay as a reference. Prior work assessing LFAs (live virus) and NAATs used the same methods.

Results: The six FDA-cleared molecular tests consistently detected 2024 H5N1 (B3.13) at 16 to 775 TCID50/swab (Table 1). In comparison, prior testing of POC molecular assays demonstrated detection at 1.55 to 7.75 TCID50/swab. Prior testing with live virus showed that 11 of 12 LFAs detected 2024 H5N1 with sensitivity ranging from 78 to 1550 TCID50/swab with one outlier that was above 1550 TCID50/swab.

Conclusions: Six FDA-cleared small footprint, sample-to-answer molecular influenza A tests consistently detected a U.S. 2024 H5N1 strain in contrived nasal swab specimens. In the event of human-to-human transmission, clinical performance and optimal sample types would need to be established for these and other clinical influenza A diagnostics.

Epigenetic Silencing of HBV cccDNA: Role of Histone H1-0 in Host Restriction and Viral Evasion

Authors: DANGAS, GEORGIOS; Moschogianni, Evgenia; Sanders, Madeleine; Athanasiadis, Antonis; Li, Dar-Yin; Maslarinou, Anthi; Levenson, Kenneth; Zou, Chenhui; Zhou, Yichen; Ogata, Kosuke; de Jong, Ype; and Michailidis, Lefteris.

Presenting Author: Georgios Dangas, BS

Background: Chronic hepatitis B virus (HBV) infections affect 300 million worldwide. A significant role in chronicity is attributed to the nuclear form of the HBV genome, termed covalently closed circular DNA (cccDNA). The cccDNA serves as the template for the transcription of all viral mRNAs, and its maintenance and stability in infected hepatocytes are the targets of curative therapies. Approved treatments against chronic HBV include nucleoside analogs and interferon-alpha (IFNα). IFNα has moderate effects on HBV replication with severe side effects. However, in ~10% of individuals who receive IFNα, this can result in a functional cure. While there is a need for IFN-free regimens, understanding the molecular mechanisms of IFNα against HBV may lead to novel antiviral strategies.

Methods: To model chronic HBV in vitro, we developed a system based on culturing mouse-passaged primary human hepatocytes (mpPHH) isolated from HBV-infected humanized mice. A significant advantage is that nearly all hepatocytes are infected and contain high levels of cccDNA. Furthermore, we established robust CRISPR-based methods in mpPHH to interrogate the impact of gene knockouts. Combined with highly sensitive proteomics analyses, we can now investigate the role of specific host factors and pathways on HBV lifecycle and IFNα activity.

Results: Infected and uninfected mpPHH displayed very distinct protein expression patterns. Forty-two proteins induced by IFNα in uninfected mpPHH were suppressed in HBV-mpPHH. These 42 hits are validated in CRISPR knockout to determine their impact on HBV infection. Understanding the mechanism of action of these proteins in terms of HBV replication is part of these efforts to identify novel druggable pathways toward eliminating/silencing the cccDNA.

Conclusions: Together, these data are expected to identify host factors crucial in chronic HBV and response to IFNα treatment. Moreover, the in vitro systems we developed, CRISPR-based applications, and systems biology analyses can be extended to other areas of HBV and liver-related diseases.

Infants' Humoral Immune Responses to Primary Influenza Vaccination and Infection

Authors: JOSHI, DEVYANI; Kumar, Sanjeev; Burrell, Allison; Bedoya, Shamika; White, Brendon; Lowen, Anice; Staat, Mary; and Wrammert, Jens

Presenting Author: Devyani Joshi, Ph.D.

Background: A major obstacle in the development of the universal vaccine against influenza is a rapidly shifting nature of the viral immune dominant epitopes. The further confounding obstacle is the antigenic imprinting, where an individual's first exposure to influenza virus can shape the humoral immune response to subsequent infections and vaccinations. Here we studied the effects of imprinting in infants. The infants were followed with weekly nasal swabs and timely blood collections beginning soon after birth, allowing us to identify symptomatic and asymptomatic respiratory infections and evaluate the immune response both prior to and longitudinally after each influenza vaccination and infection. The blood collections starting at birth also allowed us to study the effects of maternal antibodies on influenza specific response in the infants.

Methods: The IgG binding antibody responses, HAI titers and neutralizing antibody titers to various influenza viruses, including H1N1, H3N2 and B. The antibody responses were evaluated to study the magnitude and durability of maternal antibodies, impact of number of vaccinations, H1- and H3-infection, and hybrid immune response to influenza vaccination and infection.

Results: The infants had high magnitude of influenza-specific maternal antibodies at birth. These antibodies showed a fast decline and persisted for close to 9 months from birth. The infants showed an increasing antibody response with increasing numbers of yearly influenza vaccinations. However, the response showed a fast decline post vaccination. As opposed to vaccination, infants' antibody response to infection was much more durable. For the infants who received an influenza vaccination post infection, the antibody response post vaccination was strongly skewed towards the infecting strain of the virus.

Conclusion: Infants produced increasing magnitude and higher durability of antibody response to influenza vaccine with increasing numbers of yearly vaccinations. The antibody response to influenza infection is higher in magnitude and much more durable than the antibody response to 2-dose influenza vaccination as a first exposure. In case of prior influenza infection, the antibody response to influenza vaccination is skewed towards the infecting strain of the virus.

Community-Based Household Surface Sampling: A Citizen Science Approach for Recovery of Multidrug-Resistant Enterobacterales from Household Environments

Authors: Akter, Mahfuza; Cunha, Federico; Breeze, Barrett; Logan, Jaiden L.; Babbs, Catherine; Shen, Katherine; King, Etana; Shepherd Hammond, India; Herrera, Kara; Logan, Latania K.

Presenting Author: Mahfuza Akter, PhD

Background: Multidrug-resistant (MDR) Enterobacterales (Ent), particularly extended-spectrum β-lactamase (ESBL)-producing strains, pose a major global health threat and are increasingly reported from community settings. However, the role of household surfaces as reservoirs for these pathogens remains poorly defined. Identifying these reservoirs with a simple collection method is essential for understanding transmission pathways and developing effective prevention strategies.

Methods: We performed control experiments with ESBL-producing Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, and extended-spectrum cephalosporin-resistant Acinetobacter baumannii. A defined inoculum was applied to cloth, plastic, and stainless-steel surfaces, sampled with sponge sticks, and processed after 24 hours. Samples were plated on CHROMagar™ ESBL and recovery efficiency was calculated by comparing initial and final CFU counts. We then employed a citizen science approach to conduct household sampling in 13 homes targeting five high-contact surfaces. To make participation accessible, we provided households with clear, illustrated instructions for collecting samples under real-world conditions. Identification and antimicrobial susceptibility testing (AST) of sponge-stick household isolates recovered from CHROMagar™ ESBL were performed using the VITEK® 2 system.

Results: Recovery rates varied by organism and surface type, with A. baumannii and K. pneumoniae having the highest recovery (~98% on metal, 75% on plastic and 64% on cloths), while P. mirabilis consistently exhibited poor recovery. These results confirmed the reliability of sponge-stick sampling for capturing surface-associated Ent. From household samples, we recovered 34 bacterial isolates from 69% of households, including 16 Ent (47%). Identified Ent were Pantoea agglomerans, E. cloacae, and E. coli. AST revealed 31% of Ent were MDR, most notably in an E. coli isolate, which was resistant to third-generation cephalosporins, piperacillin/tazobactam, and fluoroquinolones. PCR confirmed the presence of blaCMY-2, a plasmid-mediated AmpC cephalosporinase.

Conclusions: These findings demonstrate that sponge-stick sampling is feasible for citizen scientists and effective for detecting MDR Ent in households. Our results highlight the household environment as a reservoir for MDR Ent and support community-engaged surveillance as a valuable tool for addressing antimicrobial resistance.

Household and Environmental Factors Associated with Community Spread of Multi-Drug Resistant Enterobacterales

Authors: Breeze, Barrett; Babiker, Ahmed; Konda, Sreenivas; Schneider, Alaina; Green, Stefan J.; Babbs, Catherine C. ; Cunha, Federico; Shen, Katherine Y.; Shepherd Hammond, India; Fritz, Stephanie A. ; Logan, Latania K.

Presenting Author: Barrett Breeze, BS

Background: Community-acquired multi-drug resistant (MDR) Enterobacterales (Ent) are emerging as a serious public health threat, yet the epidemiologic and environmental factors driving acquisition and transmission remain unknown.

Methods: We examined samples from 150 households of children enrolled in the SHINE study (NCT02572791) in St. Louis, MO. Epidemiological, clinical, and household data were collected, and inguinal swabs were obtained from adult and pediatric participants and up to 21 household surfaces. Swabs were enriched in tryptic soy broth, cultured on m-FC agar and CHROMagar™ ESBL, and resulting isolates were identified and tested for antibiotic susceptibility (Vitek 2™). We used PCR to detect beta-lactamase (bla) and plasmid-mediated fluoroquinolone-resistance (PMFQR) genes. Whole genome sequencing (WGS) was performed on MDR Ent from select households with >1 colonized surface and/or person(s). We conducted a retrospective case–control analysis comparing 53 households with MDR Ent to 97 households without MDR Ent using clinical metadata from participant surveys. Statistical analyses were performed to identify factors associated with household MDR Ent colonization.

Results: Of 150 households sampled, 120 MDR Ent strains were recovered from 53 households (35%). The majority were Enterobacter spp. (71%, n=85), Pantoea spp. (12%, n=14) and Klebsiella spp. (8%, n=10). Of the 76 MDR Ent selected for WGS, the most common species were E. hormaechei (n=47) and K. pneumoniae (n=10). All 76 MDR Ent were extended-spectrum cephalosporin-resistant and 10 had resistance to >1 carbapenem. All 47 E. hormaechei isolates carried a blaACT ampC variant, while K. pneumoniae primarily harbored the blaSHV-ESBL gene. Relatedness analysis found clustering of isolates within the same households from multiple surfaces and household members. In multivariable analysis, protective factors included households identifying as predominantly white and having private insurance, while factors associated with MDR Ent colonization included having a household member with ADHD, >1 child in daycare, and >1 dog in the household.

Conclusion: Households serve as reservoirs for MDR Ent, with strain clustering across people and surfaces indicating potential transmission. Factors associated with MDR Ent in households included dog ownership and daycare attendance, which underscore the need for a One Health approach to antibiotic resistance in an effort to mitigate community transmission.

Ultrasound Imaging of Draining Lymph Nodes: A Surrogate Marker for Vaccine-Induced Immune Activation

Authors: Radi, Christelle; Kalash, Suha; Dib, Serena Maria; Wimalasena, Sonia; Khalil, Lana; Kazzi, Bahaa; Graciaa, Daniel; Gromer, Daniel J.; Pulendran, Bali; Rouphael, Nadine; Al-Khafaji, Ahmed B.; Fauria-Robinson, Christian A.; and Newell, Mary S.

Presenting Author: Christelle Radi, MD

Background: Lymph node germinal center reactions are critical for generating robust immune responses to vaccines. Identifying reliable radiologic surrogates could improve vaccine evaluation by providing a non-invasive method to assess immunity. This study evaluates whether ultrasound imaging measurements of draining lymph nodes correlate with vaccine-induced immune responses, potentially offering valuable insights into the dynamics of immune activation.

Methods: Ultrasound imaging was performed on participants who received either the yellow fever vaccine (YFV; n=17), a highly durable live-attenuated vaccine, or the lower durability quadrivalent inactivated influenza vaccine (QIV; n=14). Lymph node width and cortex size were measured at baseline (pre-vaccination) and on selected post-vaccination days (Days 3, 7, 15, 21, and 29) to capture temporal changes in lymph node architecture. Continuous data were expressed as mean ± SD or median [Q1, Q3], and comparisons were made using either the paired-sample t-test or the Wilcoxon signed-rank test, depending on the normality of the data, which was assessed using the Shapiro-Wilk test.

Results: We found increases in lymph node cortex size (median change 31.0%, p=0.039, n=8) and width (median change 10.78%, p=0.099, n=17) between baseline and maximal measurement at Days 3 to 15. There was a more pronounced increase in node width in the YFV group (median change 52.94%, p=0.078, n=7), likely reflecting the stronger immune response induced by live-attenuated vaccines, whereas inactivated vaccines elicit a weaker response (median change 8.96%, p=0.77, n=10). No significant changes in lymph node dimensions were detected between baseline and other timepoints. Antibody testing is ongoing.

Conclusion: Ultrasound imaging of the draining lymph node shows promise as a surrogate marker for detecting vaccine-induced immune activation. The observed trends in structural changes suggest potential differences in immune responses between live-attenuated and inactivated vaccines. To establish the broader applicability of this technique, larger sample sizes and evaluation across a diverse range of vaccines are needed, along with comparisons to immunogenicity measurements to determine the correlation between lymphatic activation and immune response.

Community Reservoirs of Antibiotic Resistance: Enterobacterales in Green Algae from Chicago Waterways

Authors: Shen, Katherine Y.; Grayer, Danielle; Cunha, Federico; Breeze, Barrett; Logan, Jaiden L.; Logan, Jovan K.; Babbs, Catherine C.; Akter, Mahfuza; King, Etana; Yancey, Olivia; Vernon, McKenna; Medernach, Rachel L.; Silvestri, Jean; and Logan, Latania K.

Presenting Author: Katherine Shen, BS

Background: Community-acquired multidrug-resistant (MDR) Enterobacterales (Ent) infections in children and adults are rising. Our research has found that infections cluster by geographic area (neighborhood), are not linked to healthcare or antibiotic exposures, and that environmental sources such as waterways serve as reservoirs of resistance. Filamentous freshwater green algae provide nutrients and shelter that enhance Ent environmental persistence and proliferation. Our prior work found antibiotic-resistant (AR) Ent in Cladophora, a green algae, but whether increasing resistance in Ent colonizing humans and animals is similarly increasing in algae has not been studied.

Methods: We analyzed algae collected near shore from multiple high-use waterways in metropolitan Chicago to compare resistance trends among Ent harbored by algae between 2002-2025. Samples were macerated, resuspended, and processed by EPA membrane filtration, then cultured on m-FC, TBX, and CHROMagar™ ESBL agars. Ent isolates were identified using Vitek 2™ followed by antibiotic susceptibility testing (AST) via disk diffusion methods and Vitek 2™. We compared AR data from Ent in algae recovered from waterways during 2002-2003 and 2015 to Ent in algae from 2025, to determine if resistance was increasing over time.

Results: Algae from 2002–2003 harbored AR E. coli (n=160) with some resistance (tetracyclines 7.5%, cefoxitin 8%, and cefazolin 5.6%), while Chicago beach waters containing algae from 2015 exhibited increasing AR among E. coli (n=185) across broader antibiotic classes (ampicillin 12.4%, tetracyclines 8.1%, piperacillin 7%, cefazolin 3.8%. cefoxitin 3.2%, and 1.1% ceftriaxone). In 2025, we recovered 93 E. coli from algae in 15 waterways to date. AST was performed on isolates from 15 sites, and by comparison, AR prevalence in E. coli within waterways algae from 2025 demonstrated increases in resistance to ampicillin 14.0%, cefazolin 38.7%, ceftriaxone/cefotaxime 7.5% and ertapenem 1.1%. Additionally, 1.1% were MDR and 6.5% were presumptive ESBL-producing E. coli.

Conclusions: Antibiotic resistance in Ent associated with green algae in Chicago waterways has increased over time, suggesting algae may serve as an important community AR reservoir. These findings highlight a relatively unknown reservoir of AR and underscore the need for targeted public health interventions and risk communication for waterways users, in an effort to limit environmental dissemination of resistance.

Poster Number: 1

Pediatric Vaccine Hesitancy and Families Seeking Care in Urgent Care Centers

Authors: BUCHANAN, STACY; Cherven, Brooke; Dye, Betsy; Oratz, Temima; and Gettis, Margaret

Presenting Author: Stacy Buchanan, DNP, MSN

Background: Adherence with standard practices vaccinating pediatric patients remains a challenge for healthcare providers. Despite scientifically supported vaccine recommendations, some parents are hesitant or refuse to vaccinate their child. Vaccine hesitancy is defined as a refusal, delay, or modification of recommended vaccinations. The global impact of vaccine hesitancy can lead to higher incidences of communicable diseases. The purpose of the initiative: to assess parents' perceptions of their child's immunization status, barriers to vaccination, and utilization of a primary care provider (PCP). Also identify missed opportunities to intervene with parents whose child's immunizations may not be up to date.

Methods: Parents of children (ages 4 months-20 years) visiting 2 of 8 urgent care centers between May-July 2024 were invited to complete 7-item questionnaires assessing their child's immunization status, perception/barriers of vaccination, and primary care provider. Parents were approached while in the waiting room; non-English speaking caregivers were excluded. All urgent care visits for patients ages 6-21.99 years during the same period were reviewed to assess vaccination status for measles, mumps, rubella (MMR) and varicella vaccine series; vaccination status was considered incomplete if the patient had <2 doses (aligning with national age-based recommendations).

Results: Of the 76 parents eligible, 80% completed the questionnaire. Fifteen declined participation; 12 were excluded due to language barriers. Ninety-three percent of parents routinely vaccinated their child, 6% didn't. Ninety-eight percent of parents didn't decline or delay vaccines. Eighty-nine percent of parents reported a PCP. When sick, 84% of parents take their child to the PCP, 19% to the emergency department and 6% to urgent care. Most parents were uncertain if providers would refuse to see their child if parents refused vaccinations. Reasons for delaying vaccines: too many vaccines at once, prematurity, autism concerns, or immunocompromised health. Of the 10,043 urgent care encounters during the project period, 9.47% and 8.39% were incomplete for MMR and varicella vaccine series, respectively.

Conclusion: Most parents had their children vaccinated at recommended times and had a PCP. Unimmunized or under-immunized ill children seeking care may visit urgent care clinics and emergency departments. Opportunities exist to inform caregivers about the importance of vaccines.

Poster Number: 2

Antibody-dependent Zika virus infection of Human Placental macrophages occur predominantly through FcγRIA

Authors: Lingling Xu

Presenting Author: Lingling Xu

Zika virus (ZIKV) can be vertically transmitted from a pregnant mother to the developing fetus, resulting in microcephaly and/or other congenital malformations. Dengue virus (DENV)-cross-reactive antibodies can facilitate ZIKV placental transcytosis and enhance ZIKV infection of placenta macrophage-Hofbauer cells through binding to Fc- receptors (FcγR). Here, we determine the individual impact of FcγRs on antibody-mediated ZIKV infection in Hofbauer cells using two independent genetic approaches. First, by generating a comprehensive panel of Fc-variants spanning a wide range of binding affinities to different FcγRs, we find a positive correlation between binding affinity to FcγRIA and increased infection rates in pro-monocytic cell line, U937 and primary placental macrophages. Second, by genetically knock-out each individual FcγR in U937 cells using CRISPR-cas9, we determine the molecular basis of enhanced ZIKV infection by promoting both ZIKV entry and replication. These findings underscore the significance of FcγRIA in ZIKV vertical transmission and highlight the important implications for the development of strategies to prevent ZIKV transmission from mother to fetus

Abstracts for Poster Presentations

In Numerical Order

Poster Number: 3

SARS-CoV-2 EndoU-ribonuclease regulates RNA recombination and impacts viral fitness

Authors: Yiyang Zhou, Yani P. Ahearn, Kumari G. Lokugamage, R. Elias Alvarado, Leah K. Estes, William M. Meyers, Alyssa M. McLeland, Angelica L. Morgan, Jordan T. Murray, David H. Walker, Bryan A. Johnson, Andrew L. Routh, Vineet D. Menachery

Presenting Author: Yiyang Zhou, PhD

Background: RNA recombination is an important aspect of virus evolution. Coronaviruses (CoVs) maintain large RNA genomes that frequently undergo mutations and recombination, contributing to their evolution and emergence. NSP15 is a conserved coronavirus endoribonuclease (endoU). It is unknown whether NSP15 has a role in regulating CoV RNA recombination.

Methods: Using reverse genetic engineering, we created a NSP15 catalytic mutant. Using our next generation sequencing platform ("Tiled-ClickSeq") and bioinformatic pipeline, we are able to capture the landscape of CoV RNA recombination under the influence of NSP15.

Results: In this study, we find that SARS-CoV-2 has greater RNA recombination frequency than other human CoVs. In addition, CoV RNA recombination primarily occurs at uridine (U)-enriched RNA sequences. NSP15 mutant virus shows attenuated viral replication in vitro and in vivo. Next-generation sequencing (NGS) demonstrated that loss of EndoU activity disrupts SARS-CoV-2 RNA recombination by reducing viral sub-genomic mRNA but increasing recombination events that contribute to defective viral genomes (DVGs). This also leads to dysregulated inflammation in infected animal lungs.

Conclusions: The study demonstrates that NSP15 plays a critical role in regulating RNA recombination and SARS-CoV-2 pathogenesis.

Poster Number: 4

Design and Synthesis of Novel Dimeric Molecules Targeting HBV Capsid Assembly

Authors: FAN, CHIA HAN (VANESSA); Chen, Zhe; Amblard, Franck and Schinazi, Raymond F

Presenting Author: Chia-Han Fan

Chronic hepatitis B (CHB) is still a global burden which afflicts around 300 million infected individuals, resulting in more than 900,000 deaths every year, from cirrhosis, liver failure and liver cancer. Nucleos(t)ide analogs are the only approved therapeutic options. While these compounds can suppress HBV replication by inhibiting the viral reverse transcriptase, they cannot eradicate key viral markers such as cccDNA or eliminate all viral antigens (HBsAg, HBcAg). Hence, the existing treatments are curative, and lifelong continued therapy is still required.

Because of this limitation, new curative therapeutics targeting other viral proteins involved in HBV replication are being developed. The HBV capsid is an icosahedral core that plays a crucial role in the HBV replication cycle. It is involved in the co-encapsidation of viral pregenomic RNA (pgRNA) with the viral polymerase and it enables reverse transcription of the encapsidated pgRNA into relaxed circular DNA (rcDNA). Our group discovered a series of HBV capsid assembly modulators (CAMs) binding at the dimer-dimer interface of the hepatitis B core protein (HBc) and interfering with assembly of new HBV capsids. Among them GLP-26 was shown to induce a decrease in viral loads and viral antigens, both in vitro and vivo, and led to the discovery of ALG-001075 and its water-soluble prodrug ALG-000184, which is currently in Phase 2 clinical trial. More recently, we identified a novel series of dimeric HBV CAMs (D-CAMs) susceptible of acting as an inter- or intra- capsid staple. Some of these dimer exhibit better potency than their monomeric counterpart against several markers of HBV replication and display unique effects on the capsid morphology.

Based on this new discovery, novel dimeric analogs are being designed, synthesized and evaluated to increase the overall drug like properties of our first generation D-CAMs.

Poster Number: 5

Developing a Mouse Model to Assess mRNA Vaccine-induced Enhanced Respiratory Syncytial Disease

Authors: Ranjini Sankaranarayanan, Binh Ha, Heying Sun, Larry J. Anderson

Presenting Author: Ranjini Sankaranarayanan

Respiratory syncytial virus (RSV) is a leading cause of acute lower respiratory infections in infants and young children. Although globally estimated to be responsible for >100,000 deaths in children <5 years, no vaccine exists for this age cohort. A recent clinical trial of two preF mRNA vaccines in young children reported enhanced respiratory disease (ERD) in the vaccinated group compared to the placebo, despite evidence that these mRNA candidates exhibited no immunological or pathological features associated with vaccine-associated ERD after RSV challenge in mice and cotton rat models. In this study, we sought to develop a mouse model to better assess enhanced disease risk of the preF mRNA vaccine candidate. Validity of the model was determined based on its ability (a) to induce an immune response and (b) allow for viral replication in the lung, while inflammation in the lung was used as an indicator for enhanced disease. We tested lower concentrations (0.03ug, 0.1ug, 0.3ug and 1ug) of the preF mRNA vaccines in Balb/c mice with a longer interval between vaccination and RSV challenge, 7 weeks, to determine the optimal conditions for a vaccine induced inflammation model. Vector without RSV-mRNA, FI-RSV, and live RSV served as immunization controls. Our results indicated that high dose mRNA vaccine (1ug preF mRNA) was able to induce high levels of protective antibodies and effectively neutralize virus replication. Lower doses (0.03ug, 0.1ug and 0.3ug) of preF mRNA induced lower levels of antibodies with no effect on viral replication in the lung. However, these lower doses seemed to induce enhanced disease in the lung, as seen with leukocyte infiltration, upon viral challenge. Further studies with lower concentrations of preF mRNA vaccines are required to understand and prevent vaccine-induced ERD, which may aid in the development of more effective vaccines in the future.

Poster Number: 6

Discovery and Characterization of IFI44 as a Key Hepatocyte ISG Restricting Hepatitis B Virus Infection

Authors: Moschogianni, Evgenia; KARVER, KIRIN; Dangas, Georgios; Fu, Lukas; Athanasiadis, Antonis; Sanders, Madeleine; Li, Dar-Yin; Cataneo, Allan Henrique Depieri; Levenson, Kenneth; Cuba, Jens; Zou, Chenhui; Zhou, Yichen; de Jong, Ype P.; and Michailidis, Lefteris

Presenting Author: Kirin Karver

Hepatitis B virus (HBV) chronically infects over 250 million people worldwide and remains a significant cause of liver-related morbidity and mortality. Despite the availability of nucleos(t)ide analogs and interferon-alpha (IFNα), a functional cure is rarely achieved. Since IFNα exerts its antiviral effects through the induction of interferon-stimulated genes (ISGs), identifying and characterizing ISGs in hepatocytes with robust antiviral activity is essential for improving therapeutic strategies.

To identify hepatocyte ISGs with anti-HBV activity, RNA sequencing was performed on IFNα-treated humanized mice with chimeric human livers and on mouse-passaged primary human hepatocytes (mpPHHs). This comparative transcriptomic analysis revealed a set of approximately 100 ISGs that were consistently and strongly induced in both systems. A targeted CRISPR-Cas9 knockout screen in HepG2-NTCP cells was then conducted to assess the functional impact of these ISGs on HBV replication. Among the most prominent hits, IFI44 was identified as a potent restriction factor, with its knockout resulting in a significant increase in HBV infection in the presence of IFNα. This observation was consistent with prior results from an independent ISG overexpression screen, which also highlighted IFI44 and its paralog IFI44L as candidate antiviral effectors.

IFI44 has previously been associated with antiviral responses against several RNA viruses, including HCV, HIV, Dengue virus, and Influenza A virus; however, its role in HBV infection has not been previously reported. Single-cell knockout clones of IFI44 and IFI44L in HepG2-NTCP cells confirmed the CRISPR screen findings, with enhanced HBV replication observed upon IFNα stimulation, particularly in IFI44-deficient cells.

Together, these findings establish IFI44 as a robust interferon-induced restriction factor against HBV and provide new insights into the host-mediated antiviral defense landscape in human hepatocytes. Ongoing studies aim to elucidate the molecular mechanism of IFI44's antiviral activity and evaluate its therapeutic potential.

Poster Number: 7

Clinical predictors of fatality in pediatric Rocky Mountain spotted fever cases in Sonora, Mexico 2004-2024

Authors: BELLMAN, STEPHANIE; McCoy, Kaci; Enriquez, Diana; Romo, Pamela; Murray, Kristy; and Alvarez-Hernandez, Gerardo

Presenting Author: Stephanie Bellman, PhD

Background: Rocky Mountain Spotted Fever (RMSF) remains a life-threatening tick-borne disease in northern Mexico, particularly affecting pediatric populations. Despite public health measures, case fatality rates (CFRs) in this hyperendemic region have been over 3x higher than across the border in the U.S.

Methods: We conducted a retrospective analysis of 500 pediatric RMSF cases hospitalized at Hospital Infantil del Estado de Sonora (HIES) between 2004 and 2024. Clinical, laboratory, and sociodemographic data were extracted and analyzed using descriptive statistics and multivariable logistic regression to identify factors associated with fatal infections.

Results: The overall CFR was 19.8%, decreasing from 31.4% (2004-2013) to 14.5% (2014-2024). When including life-altering sequelae, the burden rose to 32.7% for the study period. Clinical presentation was non-specific with most children presenting with fever, rash, and headache. Fatal outcomes were significantly associated with delay in doxycycline treatment (>5 days after symptom onset), older age, Indigenous background, and abnormal laboratory markers such as elevated neutrophil counts, increased neutrophil-to-lymphocyte ratio, prolonged thromboplastin time, and elevated creatinine. Urban predominance and year-round incidence were observed, with no clear seasonal pattern.

Conclusions: RMSF continues to pose a serious threat to children and adolescents in Sonora. Timely administration of doxycycline remains a critical factor in reducing mortality. Vulnerable populations-including those living in poverty, children over ten years of age, and Indigenous communities-require targeted interventions. Strengthening early diagnostic capacity and developing predictive tools for severe disease could improve outcomes in endemic regions.

Poster Number: 8

Imaging HIV-1 Restriction by MX2

Authors: TORRES RIVERA, DARIANA; Betancor, Gilberto; Malim, Michael H.; and Melikian, Gregory.

Presenting Author: Dariana Torres Rivera, BS

Background: A major obstacle in the development of the universal vaccine against influenza is a rapidly shifting nature of the viral immune dominant epitopes. The further confounding obstacle is the antigenic imprinting, where an individual's first exposure to influenza virus can shape the humoral immune response to subsequent infections and vaccinations. Here we studied the effects of imprinting in infants. The infants were followed with weekly nasal swabs and timely blood collections beginning soon after birth, allowing us to identify symptomatic and asymptomatic respiratory infections and evaluate the immune response both prior to and longitudinally after each influenza vaccination and infection. The blood collections starting at birth also allowed us to study the effects of maternal antibodies on influenza specific response in the infants.

Methods: The IgG binding antibody responses to the HA proteins of various influenza viruses, including H1N1, H3N2 and B, were measured by ELISA analysis. The antibody responses were evaluated to study the magnitude and durability of maternal antibodies, impact of number of vaccinations, infection, and hybrid immune response to influenza vaccination and infection. We compared the infants' responses to a cohort of adult donors who received 2021-2022 influenza vaccination.

Results: The infants had high magnitude of influenza-specific maternal antibodies at birth. These antibodies showed a fast decline and persisted for close to 9 months from birth. The infants showed an increasing antibody response with increasing numbers of yearly influenza vaccinations. However, the response showed a fast decline post vaccination, comparable to the adults' response to influenza vaccine. As opposed to vaccination, infants' antibody response to was much more durable. For the infants who received an influenza vaccination post infection, the antibody response post vaccination was strongly skewed towards the infecting strain of the virus.

Conclusion: Infants produced increasing response to influenza vaccine with increasing numbers of yearly vaccinations. The antibody response to influenza infection is higher in magnitude and more durable than the response to vaccination. In case of prior influenza infection, the antibody response to influenza vaccination is skewed towards the infecting strain of the virus, underscoring the original antigenic sin.

Poster Number: 9

The furin cleavage sequence is not required for SARS-CoV-2 transmission

Authors: Morgan, Angelica; Vu, Michelle; Lokugamage, Kumari; Zhou, Tommy; Meyers, William; Alvarado, Rojelio; Ahearn, Yani; Estes, Leah; Plante, Jessica; Johnson, Bryan; Plante, Ken; Walker, David; and Menachery, Vineet.

Presenting Author: Angelica Morgan, BS

Background: SARS-CoV-2 emerged in 2019 and caused the COVID-19 pandemic. The SARS-CoV-2 spike is a key viral glycoprotein responsible for receptor binding and entry. The spike protein has two features that differentiate it from other group 2B coronaviruses: the presence of a furin cleavage site (FCS; PRRAR sequence) and an extended loop (17AA vs 13AA in other sarbecoviruses) with an upstream QTQTN amino acid sequence. Our prior works show that shortening the S1/S2 loop by deleting either the FCS (ΔPRRA) or upstream sequence (ΔQTQTN), ablates spike processing, changes host protease usage, and attenuates infection in vitro and in vivo. With the importance of the loop length established, here we evaluated the impact of the FCS if the S1/S2 loop length is preserved.

Methods: We utilized our established multi-plasmid reverse genetics system to generate a mutant that disrupts the FCS while preserving loop length (PQQA). Viral replication was assessed in respiratory Calu3-2B4 cells, and pathogenesis and transmission were evaluated in Golden Syrian hamsters.

Results: Our SARS-CoV-2 PQQA mutant produces similar results compared to ΔPRRA showing reduced replication, decreased spike processing, and attenuated disease in hamsters. These data indicate that loss of the FCS, not the decrease in loop length alone, attenuates SARS-CoV-2 pathogenesis. We performed a contact transmission study using hamsters and found that PQQA is transmittable despite lacking an FCS. A subsequent competition study shows severe attenuation in PQQA transmission compared to WT.

Conclusion: The data argues that the FCS is required for SARS-CoV-2 pathogenesis but not transmission. Ongoing work will evaluate the role of loop length in addition to the FCS in SARS-CoV-2 transmission. Notably, many merbecoviruses also possess an elongated loop and FCS, and we will evaluate the broader significance of those factors in another epidemic coronavirus, MERS-CoV. Additionally, we are exploring how the FCS impacts replication in zoonotic hosts. We have found that both FCS mutants (ΔPRRA and PQQA) have a replicative advantage in bat cells expressing hACE2. These results suggest host protease differences may alter the fitness advantages for the FCS in SARS-CoV-2 between bat and human cells.

Poster Number: 10

Multi-pathogen Screening of Lesion Swab Specimens Submitted for Clinical Testing at a National Reference Laboratory

Authors: Rao, Anuradha; SABINO, COURTNEY; Bowers, Heather B.; Sullivan, Julie; Bassit, Leda; McLendon, Kaleb; Talekar, Sharmila; Najjar, Joseph; Williams, Evelyn Kendall; Pollock, Nira R.; Aparicio, Carlos; Miller, Pamela; Lai, Eric; Lam, Wilbur; and Damhorst, Gregory L.

Presenting Author: Courtney Sabino, BS

Background: Infections presenting with localized or generalized lesions have a broad range of etiologies with different treatment implications. Targeted molecular testing is not always pursued for lesion swabs but is feasible using FDA-authorized assays or laboratory-developed tests (LDTs). As part of the NIH/NIBIB Independent Test Assessment Program (ITAP) for Diagnostic Mpox Lesion Panel Test Validation, we screened de-identified remnant lesion swab specimens from a reference laboratory for the primary purpose of identifying negative specimen matrix for test validation activities. This screening provided insight into the pathogens present in lesion specimens that tested negative on the originally ordered clinical test.

Methods: 346 specimens (Copan swabs in Universal Transport Media (UTM)) in which Herpes Simplex Virus (HSV)–1&2 and Varicella Zoster Virus (VZV) or Mpox were not detected at the source reference laboratory were screened for our program with at least one assay. Most specimens were further diluted 1:10 in transport media prior to testing on one or more of the following: the Xpert® Mpox assay (GeneXpert), a Treponema pallidum quantitative PCR LDT, and the Simplexa® HSV 1&2 Direct and VZV Swab Direct kits (LIAISON® MDX). Specimens producing positive results on any assay were not tested further, and 15 specimens with invalid results were excluded from further testing and analysis.

Results: For specimens originally negative for HSV and VZV, 3 discordant results (1 HSV-2+, 2 VZV+), no Mpox, and 1 T. pallidum were detected. For specimens originally negative for Mpox, 17 HSV-1+, 21 HSV-2+, 11 VZV+, 1 specimen HSV-1+ and HSV-2+, 1 specimen HSV-2+ and VZV+, 4 T. pallidum, and no Mpox were detected.

Conclusions: Although limited by dilution procedures and lack of clinical information, our data suggest that specimens primarily tested for HSV-1/2 and/or VZV rarely exhibit T. pallidum or Mpox as an alternative etiology. In contrast, more than one quarter of specimens originally tested for Mpox and found to be negative were from lesions potentially attributable to T. pallidum, HSV, or varicella infections. In part due to the emergence of Mpox as a sexually transmitted infection in the United States, multiplex NAATs for microbial causes of lesions may be clinically useful.

Poster Number: 11

Endosomal fusion of SARS-CoV-2 mediated by TMPRSS2-cleaved Spike glycoprotein

Authors MONICA CORTEZ and Gregory B. Melikyan

Presenting Author: Monica Cortez, BS

SARS-CoV-2 entry depends on binding of its Spike (S) glycoprotein to host-cell receptor, angiotensin converting enzyme 2 (ACE2). A subsequent cleavage event at the site denoted S2′ mediated by cell surface Transmembrane Serine Protease 2 (TMPRSS2) is crucial for rendering S fusion competent. In the absence of TMPRSS2, S cleavage is mediated by endosomal cathepsins (Cat L/B) that drive virus entry through an endosomal pathway. Here, we sought to characterize the role of TMPRSS2 in regulating SARS-CoV-2 entry site preference. We hypothesize that the expression of TMPRSS2 and the competing rate of virus uptake are the main determinants of the site of SARS-CoV-2 entry. A bulk virus-cell fusion assay showed that TMPRSS2 expression accelerates SARS-CoV-2 fusion, and as expected, TMPRSS2 inhibitors blocked virus fusion. By contrast, a pan-cathepsin inhibitor E64d was without effect on viral fusion. To assess whether expression of TMPRSS2 leads to direct fusion with the plasma membrane, we tracked entry of single SARS-CoV-2 pseudoviruses co-labeled with fluorescent markers of viral core, a releasable marker, and lipophilic membrane dye. Single virus particle fusion is manifested by release of a viral content marker, while the redistribution of the viral membrane marker allows pinpointing the sites of viral fusion. Retention of the lipophilic dye corresponds to fusion with the endosomal membrane, whereas loss indicates viral fusion with the plasma membrane. Surprisingly, we found that a large fraction of SARS-CoV-2 fusion (38%) occurred in endosomes of ACE2 and TMPRSS2 expressing A549 cells, with comparable kinetics between plasma membrane fusion and virus endocytosis. Future studies will investigate whether the competing rates of viral fusion and endocytosis modulate SARS-CoV-2 sensitivity to neutralizing antibodies and explore entry of variants of concern. This work was supported by the NIH R37 AI150453 grant to GBM.

Poster Number: 12

Multifaceted Mechanism of Inhibition of Enveloped Virus Fusion by Interferon-Induced Transmembrane Proteins

Authors VERMA, SMITA; Prikryl, David; Raghunath, Gokul; Markosyan, Ruben M.; Marin, Mariana and Melikian, Gregory B.

Presenting Author: Smita Verma, PhD

Interferon-induced transmembrane proteins (IFITMs) block fusion of diverse enveloped viruses with target cells, interfere with syncytiotrophoblast formation, and inhibit back-fusion of intraluminal vesicles with the limiting membrane of endosomes. The range of restricted viruses largely depends on IFITMs' localization, with IFITM1 being primarily localized to the plasma membrane and IFITM2/IFITM3 enriched in late endosomes. The IFITMs' ability to antagonize diverse membrane fusion reactions suggests a universal mechanism that likely involves modification of membrane properties. Indeed, published results support the ability of IFITM3 to arrest viral fusion at a hemifusion stage through rigidifying the endosomal membrane (increasing negative curvature, lipid order and bending modulus). Moreover, we have shown that the conserved amphipathic helix within the IFITM3 N-terminal domain is both necessary and sufficient for rigidifying the cell membranes and disfavoring viral fusion. Importantly, IFITMs interfere with viral fusion both when expressed in target cells and upon incorporation into virions (termed "negative imprinting" of virions). While the "tough membrane" model of inhibition of incoming viruses by IFITMs is commonly accepted, our recent findings suggest a different mechanism by which these proteins negatively imprint virions. First, whereas IFITM expression in cells increases the lipid order of endosomal compartments, our single virus imaging experiments reveal that, surprisingly, the lipid order of IFITM-containing virions is markedly reduced. Second, IFITM3 mutants that fail to protect cells from incoming viruses, including the IFITM3 mutant lacking the amphipathic helix, effectively reduce the fusion competence of HIV-1 upon incorporation into virions. These results highlight distinct functional consequences of IFITM incorporation into cellular vs viral membranes and, thereby, support a complex, multifaceted mechanism of virus restriction that involves: (i) rigidification of cell membranes at the sites of virus entry that captures virions at a hemifusion stage; and (ii) "negative imprinting" of virions through a yet unknown mechanism. This work was supported by the NIH R01 AI135806 grant to G.B.M.

Poster Number: 13

Breaking the Chain: Multi-Country Insights on Adolescent Social Mixing to Guide TB Prevention Strategies

Authors: BISWAS, SAMANTA; Shiiba, Machi; Shah, Sarita; Lopman, Ben; and Nelson, Kristin.

Presenting Author: Samanta Biswas, MBBS, MPH, MSPH

Background: Tuberculosis (TB) remains a leading infectious cause of death, resulting in an estimated 1.25 million deaths in 2023. Adolescents (10–19 years) are increasingly recognized as pivotal in transmission of Mycobacterium tuberculosis (Mtb), the causative agent of TB, due to their increased susceptibility to infectious pulmonary TB and extensive social networks. Our study aimed to characterize adolescent contact patterns in four low- and middle-income countries to inform TB prevention strategies that disrupt Mtb transmission.

Methods: We analyzed data from the GlobalMix study (2021–2024), which surveyed 715 adolescents across urban and rural sites in India, Pakistan, Mozambique, and Guatemala. Participants recorded daily interactions, including frequency, duration, location, and nature of interactions, in standardized social contact diaries. TB-relevant contacts were defined as prolonged (>1 hour) indoor interactions, close-proximity (face-to-face, ≤2 meters) interactions of any duration, and/or interactions involving physical contact. Comparative analyses using R software assessed variation across countries.

Results: Mean daily TB-relevant contacts were highest in Pakistan (5.6/day) and lowest in Mozambique (3.5/day). The majority of contacts occurred in households across all sites, with the highest proportion of contacts in households in Pakistan (83.5%). Outside of the household, schools were key sites of TB-relevant contact in India (25.4%), Guatemala (18.8%), and Mozambique (17.7%). Locations of social/leisure activities were key sites of TB-relevant contact in Mozambique (12.7%) and Guatemala (8.9%), as were places of worship in Pakistan (6.7%). Younger adolescents (10–14 years) reported more TB-relevant contacts than older peers (5.6 vs. 4.7, $p<0.001$).

Conclusions: Adolescent social mixing outside households varied across countries, emphasizing interventions target schools in India, Guatemala and Mozambique; social/leisure settings in Mozambique and Guatemala, and places of worship in Pakistan. By documenting how adolescents connect households, schools, and communities, this study provides essential behavioral evidence that can inform the development of interventions to curtail transmission in these age groups. With several novel vaccines in the pipeline for adolescents and adults, understanding how behavioral patterns drive transmission patterns in this group is timely and essential for accelerating global TB elimination. Conclusions and Next Steps: Our initial findings support the use of NFAT-Luc and CREB-Luc monocytes in further exploration of Ca^{2+} and cAMP signaling in tissue adaptation and stress responses. The broad signal range and ability to measure dose-responsive activation with chemical standards open broad avenues for future studies. In ongoing studies, we are migrating these cells through an airway monolayer to emulate lung APCs and testing established adjuvants and bacterial outer membrane vesicles for their Ca^{2+} and cAMP activation potential in addition to using primary patient samples.

Poster Number: 14

Modulation of Purinergic Signaling by Streptococcal Nucleotidases

Authors: Carr, Mary A; and LaRock, Christopher N

Presenting Author: Mary Carr, PhD

Streptococcus pyogenes (GAS) is an obligate pathogen responsible for more than half a million deaths each year. These high infection rates demonstrate the ability of the bacteria to effectively evade macrophage and neutrophilic killing. During infection, damaged and dying cells release their intracellular contents into the extracellular space, most notably ATP. When recognized by P2 purinergic receptors on the surface of immune cells, ATP promotes a pro-inflammatory response. Some pathogens produce nucleotidases which convert the extracellular ATP into the adenosine (Ado) through stepwise hydrolysis reactions. In contrast to ATP, Ado activates P1 purinergic receptors resulting in an overall anti-inflammatory response. GAS encodes S5nA, a poorly understood nucleotidase shown to convert ATP to Ado in vitro, but it remains unknown whether this nucleotide hydrolysis impacts GAS disease. Our preliminary experiments show that S5nA is dispensable during pharyngitis, but the nucleotidase is counterintuitively a liability for bacterial survival in a necrotizing fasciitis infection model. Since the lab's prior data show that death of skin keratinocytes by pyroptosis is critical for effective immune activation, we hypothesize that S5nA modifies the activation of P1/P2 signaling following host cell death, altering the kinetics of immune activation, but the downstream effect of S5nA's activity differs between infection sites. This project will identify how S5nA alters immune cell activation and signaling using in vitro and in vivo infection models. Successful completion of this project will show how GAS subverts immunity by targeting the activators of purinergic signaling. These findings offer novel insight into the fundamental host-microbe interactions that determine the bacterial clearance and host pathology associated with necrotizing fasciitis, an increasingly commonly GAS infection with poor prognosis and treatment options.

Poster Number: 15

Production of rhinovirus C2-specific monoclonal antibodies using hybridoma technology

Authors: SHOOTER, SAVANNAH; Devries, Mark; Gern, James; Schinazi, Raymond; Bochkov, Yury and Lee, Sujin

Presenting Author: Savannah Shooter, BS

BACKGROUND: Rhinovirus (RV) is the leading cause of the common cold and a major trigger for asthma and COPD exacerbations. Despite its widespread impact, no approved antivirals or vaccines exist. Among the three RV species (A, B, and C), RV-C is particularly significant in children, with RV-C2 frequently detected in hospitalized cases. To advance research and therapeutic development, we generated monoclonal antibodies (mAb) targeting RV-C2. These mAbs could serve as valuable tools for investigating virus-host interactions and contribute to future diagnostic and therapeutic strategies.
METHODS: BALB/c mice were immunized intramuscularly five times using the TriVax method with an RV-C2 peptide encoding the 2A protease region of the VP1 protein, combined with poly(I:C) and anti-CD40 to enhance the immune response. Following the final immunization, splenocytes were harvested and fused with P3X63Ag8.653 cells to generate hybridomas. More than 600 hybridoma clones were obtained through HAT and HT selection, followed by limiting dilution. The specificity of the clones was confirmed by ELISA and Western blot analysis. Mouse serum collected after the final immunization served as a positive control.
RESULTS: Using hybridoma technology, we successfully generated mAbs specific to RV-C2. Over 90% of the hybridoma clones secreted IgG antibodies that recognized the RV-C2 peptide. However, only 23 of these clones exhibited strong IgG reactivity against the native RV-C2 virus. In the ELISA assay, clones were considered positive if their IgG titers were tenfold higher than those of the control clone. Of these, 18 clones demonstrated strong binding affinity to the native RV-C2 virus in Western blot analysis. We are currently evaluating whether these clones possess neutralizing activity against the native RV-C2 strain.
CONCLUSIONS: RV-C2 peptide-based immunization using the TriVax method efficiently induced a high frequency of peptide-specific mAbs. However, only a fraction of these mAbs exhibited reactivity to the native virus, highlighting the critical role of conformational epitope recognition. These results underscore the limitations of linear peptide immunogens in generating broadly reactive antibodies and the necessity of rigorous screening to identify clones that recognize native viral structures. Ongoing neutralization assays will determine the therapeutic and diagnostic potential of these RV-C2–specific antibodies.

Poster Number: 16

Methods for Estimating VE Using Routine School Testing Data with Differential Testing Behavior

Authors: MOORE, AMY; Harton, Paige E.; Chamberlain, Allison T.; Rogawski-McQuade, Elizabeth T.; Dean, Natalie

Presenting Author: Amy Moore, MS

During the COVID-19 pandemic, many school systems implemented opt-in regular testing for students to track the spread of disease and detect cases early. Beyond the primary use of these testing programs as surveillance, the observational data collected from these programs can be leveraged to measure vaccine effectiveness (VE) among school-aged children. This project is specifically motivated by a data set collected by a large public school district over the course of 2 school years (2021-23). Despite the opt-in nature of the testing program permitting equal access to testing for all students, the motivating dataset shows evidence of differences in testing behavior between vaccinated and unvaccinated students, which violates the assumption of similar testing results between vaccination groups for estimating VE. To combat this issue, we explore strategies to adjust for differences in testing behavior observed over the course of the school year. We apply 2 methods for measuring VE to the observational data: a target trial emulation approach with matching of participants across vaccination groups and a test-negative design. For both of these methods, we compare losses to sample size due to study design, compare point estimates and confidence intervals for the estimation of VE, and consider additional sources of bias due to unmet assumptions for each adjustment strategy.

Poster Number: 17

CD8+ T-Cell Activation Is Associated with Partial Viremia Control after Antiretroviral Therapy Cessation in SIV-Infected Infant Macaques

Authors: COCKERHAM, CAMRYN; and Fonseca, Jairo; and Davis, Kaleaha; and Chahroudi, Ann

Presenting Author: Camryn Cockerham

HIV disease progression in children occurs more rapidly than in adults. Children experience unique challenges with adherence to antiretroviral therapy (ART), making a pediatric-specific cure for HIV critical. We have previously tested the combination of viral vector-mediated delivery of a broadly neutralizing antibody (eCD4-IgG1) with latency reversal (using the non-canonical NF-kB activator AZD5582) on infant SIV-rhesus macaques on ART. Following this combination intervention, ART was interrupted to assess viral rebound dynamics. Most macaques rebounded quickly; however, two intervention infants exhibited partial viremia control off ART. This project evaluates CD4+ and CD8+ activation levels in controllers vs non-controllers and assesses differences between antigen-activated cells in the partial controllers vs non-controllers.

PBMCs were collected following the intervention but prior to ART interruption and 13 weeks after ART interruption to evaluate the levels of CD4+ and CD8+ T-cell activation in animals that controlled viremia vs those that did not, and to assess differences between antigen-activated cells in the intervention and control groups. An Activation-Induced Marker (AIM) assay was used to assess T-cells stimulated with SIVgag peptide pools and stained with monoclonal antibodies to quantify cell-surface levels of CD69, CD25, OX40, and 41BB. Flow cytometry data was analyzed using Flowjo and a gating strategy following Boolean logic with the "OR" operator. Statistical analysis was conducted with GraphPad PRISM.

Levels of SIV-specific CD4+ and CD8+ T-cell activation did not differ between the control and intervention groups at either time points analyzed (prior to and during ART interruption). However, the two animals that exhibited transient post-ART control of viremia had a significantly higher level of activated CD8+ T-cells following SIVgag peptide stimulation compared to non-controllers in the intervention cohort ($p=0.0357$, Mann-Whitney U-test). CD4+ T-cell activation was similar between groups at both time points. The high levels of antiviral CD8+ T-cells prior to ART interruption may have been responsible for the partial viremic control. Our results extend to the pediatric setting, prior research indicating a role for CD8+ T-cells in post-ART and post-intervention control of viremia. Future HIV cure approaches for children should utilize these findings and focus on enhancing antiviral CD8+.

Poster Number: 18

Modifying PF74 Improves Anti-HIV-1 Activity Against the Resistance-associated Capsid Mutation N74D

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Presenting Author: William McFadden, BA, BS

Background: Lenacapavir (LEN) is an FDA-approved drug for highly treatment-experienced individuals living with multidrug-resistant HIV-1 infection. LEN inhibits HIV-1 replication with sub-nanomolar potency by targeting the capsid protein (CA) at the "FG-binding pocket" (FGBP). Clinical trials report the emergence of CA mutations like M66I and N74D after LEN treatment. N74D is also selected by PF74, the first reported antiviral to bind the FGBP with sub-micromolar potency. PF74 contains three aromatic moieties, R1, R2, and R3; our structure-based studies have developed improved compounds with unique chemistry at R1 and R3.

Methods: The EC50s of selected FGBP-targeting compounds were determined against N74D NL4-3 HIV-1 in TZM-bl cells and compared to reported wild-type (WT) potency. X-ray crystallography solved novel structures of FGBP compounds complexed with a native WT CA or with only the N74D mutation and were compared to reported structures. Thermal shift assays, analyzed with TSAR, determined the melting temperature (Tm) and biolayer interferometry determined binding kinetics to disulfide-stabilized CA hexamers (CAHEX). Transmission electron microscopy and in vitro assembly assays determine the compounds' effects on WT or N74D CA lattice.

Results: While N74D virus caused PF74 resistance, modifications at R1 and R3 improved inhibition; specifically, compounds with a para-Cl on R1 and/or N-ethyl on R3 decreased N74D resistance. Additional interactions between modified R1 and/or R3 with CAHEX increase the Tm for both WT and N74D CAHEX, more so than PF74. CA rapidly polymerized in vitro, even in the absence of salt, after the addition of compounds with R3 C5-OH modifications, likely due to a H-bonding network involving the modified indole and the adjacent CA monomer of CAHEX.

Conclusions: Understanding the interactions responsible for increased potency and decreased resistance are essential for next generation drug design. We report modifying R1 and R3 of PF74 as distinct strategies to overcome N74D resistance. One modification, R3 C5-OH, forms intermolecular interactions within and between CA subunits, which, unlike PF74, enables rapid lattice assembly in vitro. Overall, these data provide a structure-guided approach to improve antiviral activity and the resistance profile of FGBP-targeting compounds.

Poster Number: 19

SARS-CoV-2 Priming Exacerbates Influenza Severity and Mortality

Authors: MEENAKSHI KAR, Shilpi Jain, Katharine Floyd, Stephanie L. Foster, Jacob Vander Velden, Jacob Kohlmeier, Mehul S. Suthar

Presenting Author: Meenakshi Kar, PhD

Respiratory virus co-infections, particularly with SARS-CoV-2 and influenza, have been linked to worsened disease outcomes in humans, including increased hospitalization, mechanical ventilation, and mortality. However, the impact of prior SARS-CoV-2 infection on subsequent influenza virus pathogenesis remains incompletely defined. Using a B.1.351 SARS-CoV-2 mouse model, we investigated the effect of sequential SARS-CoV-2 and influenza infection at varying intervals post-SARS-CoV-2 clearance (7, 14, 21, 28, and 180 days). Lung viral loads, histopathology, innate immune cell recruitment, cytokine production, and alveolar macrophage activation were assessed. We found that prior SARS-CoV-2 infection creates a transient window of immune dysregulation during recovery that predisposes to worsened influenza outcomes. Mice infected with influenza at later recovery timepoints (~28 days) exhibited exacerbated disease and impaired neutrophil and eosinophil recruitment compared to influenza-only controls. This heightened susceptibility was associated with delayed resolution of inflammatory gene expression programs and sustained MHC-I/II and CD86 expression in the alveolar macrophages. These findings provide mechanistic insight into clinical reports of severe disease in SARS-CoV-2/influenza co-infections and highlight the importance of timing in mitigating risk.

Poster Number: 20

Modeling West Nile virus infection and host response in human brain organoids

Authors: MALAKAR, SHILU; Wariyar, Supriya Suresh; Andersen, Jimena and Suthar Mehul S.

Presenting Author: Shilu Malakar, PhD

The emergence of neurotropic arboviruses presents a significant global health threat, demanding biologically relevant models to study viral pathogenesis and evaluate countermeasures. West Nile virus (WNV) is a leading cause of Flavivirus-induced encephalitis in the United States. Although substantial progress has been made in elucidating WNV biology and pathogenesis, effective therapeutic agents and prophylactic vaccines for human application remain to be developed. WNV infects the central nervous system (CNS), yet its precise neurotropic mechanisms remain poorly understood due to limitations in current model systems. Leveraging human induced pluripotent stem cell (hiPSC) technology combined with three-dimensional (3D) culture methods, we gain a unique opportunity to model previously inaccessible human neurobiology. We developed a novel human region-specific cortical organoid model to investigate WNV neurotropism and host immune response. These 3D cellular structures closely recapitulate the cytoarchitecture and cellular complexity of the human cerebral cortex, providing a platform that offers significant advantages over traditional cell cultures or non-human animal models for studying human viral infections. Cortical organoids were infected with WNV across a dose range (105, 106, and 107 PFU), and infection progression was monitored over 14 days. Preliminary results confirmed a successful and sustained viral infection, shown by a dose- and time-dependent increase in viral load quantified by real-time RT-qPCR and standard virus titration assays. Furthermore, RT-qPCR analysis of key antiviral genes confirmed the cortical organoid's robust innate antiviral capacity following WNV infection, demonstrating significant upregulation of innate immune components (RIG-I, IRFs, type I/III interferons, and ISGs). These results establish the human cortical organoid as a valuable model for studying WNV neurotropism and the primary cell-intrinsic neural immune response in the absence of the microglial or the peripheral immune cell influence.

Poster Number: 21

From Transmission to Taxonomy: Delineating Triatoma sp. nov., a Novel Species of Chagas Disease Vector from Northern Belize

Authors: WEIMER, KATHLEEN; Gunter, Sarah; Beatty, Norman; de Oliveira, Jader; Justi, Silvia; Mendez-Cardona, Sergio; and Murray, Kristy

Presenting Author: Kathleen Weimer, MSc, PhD

Chagas disease is the leading cause of non-ischemic cardiomyopathy in the Americas, where >6 million people are estimated to be infected. Caused by the parasite Trypanosoma cruzi, transmission most often occurs through contact with the feces of triatomine vectors deposited near the bite site. Triatoma dimidiata sensu lato is considered the prevailing vector taxon of human health importance in Belize, where vectorial transmission of Chagas disease was reportedly interrupted in 2015. However, following identification of an acute case of Chagas disease in March 2020, molecular analysis of Triatoma sp. collected around the case-patient's home detected T. cruzi and Discreet Typing Units corresponding to that of the infected patient, implicating vector-borne transmission. Notably, molecular identification revealed only 95% identity to the predominating T. dimidiata - indicating emergence of a taxon not previously described. Here, we describe this newly identified taxon, Triatoma sp. nov. Morphological description was carried out with 6 collected specimens (four males and two females) from the Corozal District of Belize and compared to T. dimidiata from Central America and specimens from Belize featured in the Florida State Collection of Arthropods. Genetically, T. sp. nov. is most closely related to T. huehuetenanguensis, Lima-Cordón & Justi 2018. The new species boasts several distinguishing characteristics including a dark ventral abdomen, larger overall size, extended head length, and greater width of the pronotum. Further defining this species, scanning electron microscopy of eggs revealed a distinct symmetric ellipsoidal shape lacking a neck or collar, with a narrow ring-shape band forming a "chorial edge" adjacent to the plane of the operculum and the shell. Given the changing vector landscape within Belize and the region, it is essential to reevaluate the risk of Chagas disease to humans: identification of this novel species is an important step in understanding the current potential for vector-borne T. cruzi transmission.

Poster Number: 22

Impact of Freeze-Thaw Cycles on Detection of Respiratory Syncytial Virus (RSV) Antigens and RNA When Assessing Novel Multiplex Diagnostic Assays

Authors: HEATHER B. BOWERS, Courtney Sabino, Julie Sullivan, Eric Lai, Wilbur Lam, Raymond F. Schinazi, Leda Bassit and Anuradha Rao

Presenting Author: Heather B. Bowers, BS

Background: New 4-plex tests are being manufactured where RSV detection is included. Our studies examine RSV antigen and RNA stability using (a) RSV samples diluted in nasal wash matrix, frozen, and subjected to multiple freeze thaw cycles, and (b) RSV samples spiked on dry swabs and frozen at -80□C, then subjected to freeze thaw cycles. Here, we describe (1) RSV RNA and antigen stability when subjected to five freeze thaw cycles. (2) Determination of limit of detection (LOD) using contrived RSV samples with a novel 4-plex LFA. (3) Determination of LOD using contrived RSV samples with Cepheid Xpert Xpress CoV-2/Flu/RSV, a commonly used POC nucleic acid-based assay.

Methods: Samples were prepared by serially diluting live RSV obtained from BEI in nasal wash matrix. (1) RNA was isolated from each sample and RSV matrix (M) gene Ct was determined as a marker for RNA stability. Samples were then subjected to 1 to 5 freeze thaw cycles. RNA levels were determined after each freeze thaw cycle and compared to original values. (2) samples at 1st, 3rd and 5th freeze thaw cycles were tested with a new 4-plex LFA. (3) contrived RSV samples were spiked to swabs. These swabs were tested using Cepheid Xpert Xpress, and LOD established. (4) Sample spiked swabs were also used to determine LOD of 4-plex LFA. (5) to simulate dry swabs from patients, swabs were spiked and frozen at -80C. After freezing for >2 months, swabs will be subjected to freeze thaw experiments and tested with the 4-plex LFA and Cepheid Xpert Xpress assay.

Results: We have determined (1) Contrived RSV samples in saline are stable when stored at -80□C for two years. (2) After 4 freeze thaw cycles, the M gene Ct were comparable between all saline samples irrespective of number of freeze thaws. (3) There is no loss of antigenicity and positive LFA results were obtained from saline samples subjected to 3 or 5 freeze thaw cycles. (4) The LOD for Cepheid Xpert Xpress assay was determined to be 1.74X103, and 1.39X104 TCID 50/ml for the 4-plex LFA. (5).

Conclusions: RSV samples prepared in nasal wash appear to be stable frozen and when subjected to freeze-thaw cycles allowing long term storage of contrived eluted samples. Our ongoing studies will establish the stability of dry RSV positive swabs and will establish whether patient derived samples are best stored eluted in saline, or as dry swabs.

Poster Number: 23

Assessing Opt-Out HIV Testing in Pediatric Emergency Departments After Two Years of Implementation

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Presenting Author: Sandy Francois, MS

In July 2023, Children's Healthcare of Atlanta (Children's) progressively implemented opt-out HIV testing in its emergency departments (ED) for patients ≥13 years undergoing venipuncture at all 3 of their sites. This is in accordance with the Centers for Disease Control and Prevention recommendation that all patients ≥13 years receive HIV screening. Parts of metro Atlanta have HIV positive rates 8-times the national average with adolescents being the least likely group to know their HIV status. In the past two years, Children's HIV testing numbers have more than doubled and 12 adolescents living with HIV (ALHIV) were identified. The objective is to review characteristics of these ALHIV and identify opportunities for improving the initiative since the implementation.

Children's electronic medical record EPIC was used to evaluate the 12 ALHIV. Provider (physician/nurses) adherence to reading opt-out testing statements and the percentage of eligible patients tested (PEPT) were assessed to identify potential barriers and opportunities for improvement. Data was compared using descriptive statistics.

All 12 ALHIV were evaluated, 3(25%) girls and 9(75%) boys. The mean age (± standard deviation) was 16.0 ± 1.76. In our ALHIV, 50% were coinfected with other sexually transmitted infections (STIs): 83% chlamydia, 33% herpes simplex virus, 33% syphilis, 33% trichomonas and 33% gonorrhea. Geographically 92% of positives results were from metro Atlanta with the rest being from a rural county. The opt-out statement was read at a rate of 8%, 11% and 36% per site; the corresponding PEPT was 16%, 53% and 11%.

Atlanta remains a hotspot for new HIV cases. Twelve cases in 24 months highlight the importance of universal HIV testing of adolescents and reflects a public health crisis. The majority of our ALHIV were co-infected with STIs, emphasizing the need for integrated comprehensive preventative services. To support this initiative, it is imperative that we improve provider comfort with screening and adherence to reading opt-out testing statements through site specific interventions to ensure eligible patients are captured. ALHIV will likely be identified at an earlier stage of infection, facilitating timely access to medical care.

Poster Number: 24

A phase 1 study of inactivated rotavirus vaccine CDC-9 in healthy adults

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Background: Rotavirus is a leading cause of diarrheal morbidity and mortality in children <5 years of age worldwide. A more immunogenic rotavirus vaccine could improve effectiveness in low- and middle-income countries where disease burden is greatest.

Methods: We performed a phase 1, randomized, double-blinded, placebo-controlled (4:1 vaccine to placebo) clinical trial of a heat-inactivated, alum-adjuvanted rotavirus vaccine CDC-9 administered intramuscularly at two sequential dose levels (3.75 µg and 7.5 µg) in healthy U.S. adults 18-45 years of age. Participants were vaccinated on days 1, 29, and 57. They reported solicited local and systemic adverse reactions for 7 days following vaccination and are being followed through 6 months after completing the 3-dose series for safety and immunogenicity.

Results: To date, we have completed enrollment of 25 participants in the low-dose (3.75 ug) cohort and followed through 1 month after completing the 3-dose series. The study remains blinded. Participants are 60% female, 68% White, 16% Black, 8% Asian, 4% Native Hawaiian or other Pacific Islander, and 4% multiracial, with 8% Hispanic ethnicity and median age of 28 years (range 22-45 years). Overall, the study product has been safe and well tolerated. Common local solicited reactions included injection site pain (58%) and tenderness (68%), while systemic reactions included headache (42%) and fatigue (32%); all were mild or moderate in severity. The most common laboratory abnormality was any decrease in hemoglobin from baseline (74%), which was attributed to phlebotomy, followed by mild thrombocytopenia (26%) post-dose 3. Three participants met individual halting criteria, including one with an unrelated SAE of renal laceration, one whose contraception method expired, and one who experienced a related grade 3 neutropenia in the setting of an initial grade 1 neutropenia at baseline screening. Unrelated unsolicited AEs occurred in most participants, the most common of which was upper respiratory infection (26%). There have been no related SAEs or unanticipated problems.

Conclusions: Interim data suggests the low-dose inactivated rotavirus vaccine appears safe and well tolerated. The high-dose cohort is actively enrolling, and a future study of the CDC-9 vaccine administered intradermally by dissolvable microneedle patch is in the planning stages.

Poster Number: 25

Experimental CD8 cell depletion induces viral reactivation in ART-suppressed SIV-infected rhesus macaque infants

Authors: ZAKI POUR, SHAHAB; Colvin, Alora; Hamid, Riri Rizkianty; Jeffrey, Lifson; Keele, Brandon; Silvestri, Guido; Chahroudi, Ann; and Mavigner, Maud

Presenting Author: Shahab Zaki Pour

Background: While CD8+ T cells have been implicated in controlling HIV persistence during antiretroviral therapy (ART) in adults, their activities on HIV reservoir in infants are largely unknown. Given the distinct features of the developing immune system, we conducted a pediatric study to assess the impact of experimental CD8+ T cell depletion on the viral reservoir in ART-treated perinatally SIV-infected rhesus macaque (RM) infants.

Methods: Sixteen RM infants were infected intravenously with SIVmac239M and initiated on ART 4 weeks post-infection. After >3 months of plasma viral load (PVL) suppression on ART, animals are divided in 2 groups: 6 RMs are maintained on ART only and serve as controls while 10 RMs receive the experimental treatment consisting of a dose of the anti-CD8α depleting antibody, MT807R1, at 50 mg/kg alone or in combination with 5 weekly doses of the latency reversing agent, AZD5582, at 0.2 mg/kg. A comprehensive assessment of clinical and immunovirological parameters was performed.

Results: The experimental treatment was completed in 7 RM infants thus far and none experienced adverse events. The depletion of >99% of peripheral CD8+ T cells was followed by on-ART viremia >60 copies of SIV RNA per ml of plasma in 2/4 RMs treated with MT807R1 and 3/3 RMs treated with MT807R1+AZD5582. The frequency of viremic episodes was higher in the combined treatment group as compared to the CD8 depletion only group (28% vs 83%, P=0.0001). In the combined treatment group on-ART viremia was sustained for up to 4 weeks reaching 25,000 copies of SIV RNA per ml. A slow reconstitution of the peripheral CD8+ T cell compartment was observed starting at day 21 for the combined treatment group and day 28 for the CD8 depletion only group. In both groups, memory CD8+ T cells were the first cells to be detected in the blood.

Conclusions: Experimental CD8+ T cell depletion alone or with AZD5582 induced viral reactivation in ART-suppressed SIV-infected RM infants, suggesting that CD8+ T cells are involved in controlling viremia on ART in infants. Analyses of the viral reservoir size and clonality will further define the role for CD8+T cells in pediatric HIV persistence.

Poster Number: 26

Antibodies produced after infection with WNV-1 have reduced neutralizing ability against WNV-2

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Mosquito-borne flaviviruses cause over 100 million infections each year and are among the most rapidly spreading vector-borne pathogens. West Nile virus (WNV) is the leading cause of arboviral disease in the United States and of arboviral encephalitis in the world, but there are no approved therapeutics or vaccines for human use. The envelope (E) protein is the surface membrane glycoprotein of WNV and is essential in virion integrity as well as mediating host cell entry through receptor binding and membrane fusion. Protective immunity against WNV is dominated by antibodies targeting the E protein, preventing viral entry by blocking either attachment to the cell surface or membrane fusion. However, there are more than 30 amino acid differences in the envelope (E) protein between lineage 1 WNV (WNV-1) and lineage 2 WNV (WNV-2) that may threaten long-term protective immunity. To determine the cross-protective strength of antibody responses between WNV lineages, we leveraged a longitudinal cohort of United States blood donors who tested positive for WNV-1 and returned to provide 7 additional blood samples up to 1 year. We measured the neutralization ability of their polyclonal serum using a reporter virus particle (RVP) system based on a WNV-1 strain from the United States and a WNV-2 strain from Italy. We found that the RVPNT50 titers, the reciprocal serum dilution at which the RVP infectious units are reduced by 50%, were 1472 against WNV-1 and 273 against WNV-2. In WNV-1-infected individuals, the neutralizing ability of polyclonal sera was reduced by 5.4-fold against WNV-2 RVPs compared to WNV-1 RVPs. This level of reduction poses a challenge for successful vaccine development, showing the importance of a WNV vaccine that elicits broader immunity.

Poster Number: 27

Anti-SIV Env RhmAbs and venetoclax during analytical therapeutic interruption as a HIV-1 cure strategy in adult rhesus macaques

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Background: Although antiretroviral therapy (ART) has improved disease outcomes and reduced transmission of Human Immunodeficiency Virus (HIV-1), it requires lifelong adherence, as interruption leads to rapid viral rebound due to the latent reservoir. Also, the anti-apoptotic molecule BCL-2 supports the maintenance of CD4+ T cell reservoir during HIV-1 infection. Broadly neutralizing antibodies that target the HIV-1 envelope show great promise as a key element in HIV-1 cure strategies. So, we hypothesized that combination of a cocktail of monoclonal neutralizing antibodies that target CD4 binding site (ITS 103.01), glycan shield (ITS 90.03) and Env apex (PGT 145-R100aS) along with venetoclax a potent BCL-2 inhibitor given during analytical therapeutic interruption of ART would reduce the size of the viral reservoir.

Methods: 24 adult rhesus macaques (RMs) were infected with SIVmac239M. ART was initiated 4 weeks post-infection. Study arms included 10 ART alone controls, 7 RhmAb (ART+RhmAbs) controls, and 7 intervention (ART+RhmAbs+venetoclax) RMs. After 67 weeks of infection, ART was interrupted. RhmAbs (20 mg/kg, SC) were administered and two days later ART was interrupted for ART + RhmAbs controls, and ART + RhmAbs + venetoclax intervention animals. Five days following ATI, intervention group has received 300 mg of venetoclax (Oral route) thrice a week for two consecutive weeks. On-ART viremia measured by qPCR of SIVgag RNA throughout the study. Lymphocyte changes were assessed by flow cytometry.

Results: Following analytical treatment interruption (ATI), viral rebound in the ART alone controls occurred between 7 and 16 days with a median of 15 days. Viral rebound in ART + RhmAbs controls occurred between 41 and 153 days, with a median of 75 days, whereas in the intervention group, rebound was observed between 77 and 177 days, with a median of 112 days. Blood, bone marrow, lymph node, and rectal biopsies were collected at baseline, post-intervention, and 8 weeks into analytical treatment interruption (ATI). We anticipate a significant reduction in viral load across multiple compartments in animals from the intervention group compared to controls.

Conclusions: Treatment with venetoclax in the presence of SIV-Env-specific RhmAbs resulted in a significant delayed time to viral rebound.

Poster Number: 28

Variation among DENV1 major lineages affects neutralization by sera from naturally infected and vaccinated individuals

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Dengue virus (DENV) is estimated to cause 390 million infections annually, posing a significant global health burden with currently only limited treatment options available. DENV is comprised of four antigenically and genetically distinct serotypes (DENV1-4). Each serotype contains multiple genotypes, with major and minor lineages within each genotype defined based on phylogenetic distance. Prior studies have shown that neutralization by monoclonal antibodies can vary across genotypes within a serotype. Comparable genotype-dependent differences have also been observed in serum from TV003 recipients, particularly against DENV2. However, immunity elicited by natural infection or vaccination against DENV1 diversity remained uncharacterized. We hypothesized that diversity in the DENV1 envelope protein contributed to differential neutralization by sera from naturally infected and vaccinated individuals. To test this, we generated a panel of recombinant DENV1 reporter virus particles (RVPs), representing approximately 74% of the global genetic diversity in the DENV1 envelope protein and assessed neutralizing antibody responses elicited by natural infection and vaccination. We analyzed serum samples from individuals in a DENV1-endemic region and from recipients of the TV-003 vaccine compared to non-vaccinated and DENV-naïve individuals as controls. Preliminary analyses showed distinct neutralization profiles across the RVP panel: monoclonal antibodies displayed narrow specificity, polyclonal sera from DENV1-infected individuals showed broader reactivity, and TV003-induced antibodies exhibited intermediate responses across major lineages. We concluded that vaccine-induced antibodies exhibited reduced potency against DENV1 lineages genetically distant from the vaccine strain, whereas polyclonal sera from naturally infected individuals had broader neutralization across the panel. These findings have important implications for future vaccine development, challenging the assumption that vaccination with a single representative strain per serotype can protect against all intra-serotypic diversity. Future directions will include investigating how DENV genetic diversity impacts the durability and breadth of protective immunity from both natural infection and vaccination.