Special Issue: Abstracts From the 2024 Conference on Retroviruses and Opportunistic Infections

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About This Issue

This issue of Topics in Antiviral Medicine is a special issue that includes the abstracts from the 2024 Conference on Retroviruses and Opportunistic Infections (CROI). This issue is funded and supported by IAS–USA. Information on citing presentations from CROI 2024 is available on page 3.

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Contact Information

If you have any questions, please contact the IAS–USA.
Email: journal@iasusa.org
Phone: 415-544-9400
Mail: IAS–USA
131 Steuart Street, Suite 500
San Francisco, CA 94105

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CROI 2024 Resources

Resources from CROI 2024 can be found at www.CROIconference.org. The CROI 2024 Resources page includes the following resources and more.

The CROI Program and Information Guide includes information about sessions, speakers, and other details about CROI 2024. In addition to the Special Issue of Topics in Antiviral Medicine™, abstracts from CROI 2024 can be viewed in the Abstract eBook and the searchable database. Beginning April 6, 2024, plenaries, interactive sessions, oral abstract presentations, and poster abstract presentations will be available as webcasts. Archived webcasts from CROI 2014 to 2023 are also available. Visit the resources page of www.CROIconference.org for more details.
ABSTRACTS

Note: The data in these abstracts were current at the time they were submitted, which may have been months before the start of CROI. For the most up-to-date data, please view the posters and oral abstract presentations.

To search for specific keywords, presenters, or authors on this document, hold the “Ctrl” and press the “F” keys on your keyboard (or “Command” and “F” keys for some Apple devices) to prompt a word search.

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How to cite the abstracts:

INVITED SESSION PRESENTATION SUMMARIES

1 Overview of the Scott M. Hammer Workshop for New Investigators and Trainees
Serena S. Spudich
Katharine J. Bar
Background: Over the past four decades, remarkable progress has been made in understanding HIV epidemiology, pathogenesis, treatment, and prevention from the combined efforts of community members, clinicians, investigators, and funding agencies worldwide. Yet more work and new approaches are needed to achieve the ambitious goal of ending the epidemic and ensuring optimal quality of life for those living with HIV. To encourage and stimulate the next generation of investigators, the CROI Program Committee organizes an annual Workshop for New Investigators and Trainees comprised of expert and comprehensible talks to introduce key topics in basic, clinical, and public health investigation into HIV and related infections and to highlight relevant work to be presented over the ensuing days at CROI. This year, the program will begin with a presentation by Mr Adam Castillejo, an advocate and activist who will provide a community perspective on HIV cure. Dr Frank Kirchhoff will provide an overview of the molecular virology of HIV-1 and SARS-CoV-2 and describe key related presentations at CROI. Dr Elizabeth Connick will cover the immune responses against HIV and SARS-CoV-2. Dr Afam A. Okoye will review advances in preclinical and clinical approaches for HIV remission or eradication. Dr LaRon E. Nelson will address advances in different strategies for preventing HIV transmission. Dr Jennifer Jao will provide an overview of key topics in maternal-child HIV and highlight relevant work presented at CROI. The workshop will end with a presentation by Dr Jeanne M. Marrazzo, Director of the National Institute of Allergy and Infectious Diseases at the NIH, who will discuss opportunities in and provide insights on careers in research and discovery, reflecting on her personal career journey to date. By completing the workshop, attendees will have achieved a head start toward maximizing the knowledge gained and research ideas as they navigate CROI 2024.

2 Cryoelectron Microscopy-Based Polyclonal Epitope Mapping (cryoEMPEM)
Gabriel Ozorowski
The Scripps Research Institute, La Jolla, CA, USA
Background: Electron microscopy polyclonal epitope mapping (EMPEM) is a powerful technique for rapidly mapping the epitopes of serum antibodies and providing a visual readout of immune responses. EMPEM is performed using blood samples from immunized animal models, human volunteers, or convalescent patients of recent viral infection. Recently, our group has applied high resolution cryoEM methods to the same samples (cryoEMPEM) to visualize the amino acid interactions between antibodies and antigen. This approach can also be used to predict the sequences of the observed antibodies. By integrating NGS data of B cell repertoires with cryoEMPEM data, we are able to rapidly identify and provide unprecedented molecular resolution of epitope specific polyclonal antibody responses. EMPEM studies are complementary to traditional serological approaches, which altogether inform on the types of epitopes targeted, the diversity of epitopes targeted, and the consistency of epitopes targeted between study volunteers. When applied to HIV vaccine research, the molecular details revealed by this approach can confirm whether epitope specific, on-target antibodies are present in the sera and if they contain any structural and genetic features of known broadly neutralizing antibody precursors.

3 A Spotlight on HIV: Visualizing Post-Entry Events by Correlative Light and Electron Microscopy
Barbara Müller
University Hospital Heidelberg, Heidelberg, Germany
Background: The post-entry phase of HIV-1 replication – from cytosolic entry of the viral capsid encasing the genomic RNA to the integration of the reverse transcribed viral cDNA into the host cell genome - has long represented an enigmatic part of the viral replication cycle. During this phase, subviral particles need to undergo a complex sequence of transitions in composition and structure, which is challenging to unravel using traditional bulk virological and biochemical approaches. Direct visualization of incoming
viral structures in the cytosol by electron microscopy has been hampered by the difficulty to detect and identify rare, small objects with unknown morphology, which are embedded in a vast and complex cytosolic environment of similar electron density. Correlative Light and Electron Microscopy (CLEM) addresses this problem: rare, defined objects of interest are localized within a complex environment via fluorescence-based detection and subsequently analyzed with high spatial resolution using electron microscopic approaches. Today, continuous methodological advances allow us to analyze the morphology of virus-derived complexes within their intracellular environment in unprecedented detail. Application of this approach to HIV-1 post-entry yielded new insights that, in conjunction with other results, identify the mature capsid as a key organizer of post-entry events. The presentation will introduce the CLEM approach and highlight insights into post-entry events in HIV-1 replication, from the visualization of cytoplasmic reverse transcribing complexes in infected cells approximately 20 years ago to recent high resolution in situ imaging of capsid-like structures in transit through the nuclear pore.

**Single-Cell Multi-Omics of HIV Cellular Reservoirs**

Kevin Welther
Duke University, Durham, NC, USA

**Background:** The epithelium is a nessential first barrier against viral infection. Understanding the interactions that enable pathogens to cross this complex barrier is critical to treating a wide range of diseases, including the recent SARS-CoV-2 pandemic. Single-virus tracking (SVT) is a potentially powerful tool to capture the molecular scale details of viral infection in live cells. SVT typically relies on fluorescence microscopy and can provide different insights from ensemble bulk experiments. However, the reliance on traditional fluorescence microscopy techniques has limited the application of SVT to monolayer cell cultures with poor temporal resolution upon expansion to three-dimensional tissue models. In this presentation, we will detail current SVT methods and their advantages and limitations. We will then introduce a new active-feedback SVT method for overcoming the current limitations of SVT. This new method, called 3D Tracking and Imaging (3D-TrIm), uses fast, real-time measurement to "lock on" to a single virion and measure its dynamics at kHz or faster sampling rates across large three-dimensional spatial scales in live cells. We will demonstrate how 3D-TrIm captures transient viral contacts with the cell surface with millisecond temporal resolution, and how this new technique can translate SVT from simple monolayer cell culture models to more complex three-dimensional tissue models.

**Single-Cell Multi-Omics of HIV Cellular Reservoirs**

Iain C. Clark
University of California Berkeley, Berkeley, CA, USA

**Background:** HIV persists indefinitely within tissue and blood cell reservoirs, necessitating life-long antiretroviral therapy (ART). Single-cell omics technologies represent a promising approach to understanding cells that harbor provirus, including the unique cell-intrinsic mechanisms that promote cell survival, proliferation, immune evasion, or HIV silencing. However, despite technological advances in single-cell analysis, there are several unique challenges to using these tools in HIV cure research. First, HIV-infected cells are rare in vivo, which necessitates the single-cell sequencing of hundreds of thousands of cells per sample, at great expense. Second, HIV accumulates in heterochromatin and may not express HIV RNA. Methods like scATAC-seq or scRNA-seq therefore only capture HIV+ cells with transcriptionally active provirus and those in regions of open chromatin. Lastly, over 95% of HIV provirus in people on long-term ART are defective. Any method that seeks to understand cells with replication-competent virus must first identify the even rarer cell population that contains intact provirus. No technology has yet to address all of these challenges, but in recent years commercial scRNA-seq and scATAC-seq platforms have allowed core researchers to generate exciting datasets that begin to reveal how HIV persists during effective ART. These datasets, owing to the rarity of HIV+ cells, are large but contain only hundreds of HIV+ cells, presenting additional challenges during bioinformatic analysis and interpretation. Recently, we reported a custom droplet microfluidic technology, FIND-seq, for sorting HIV+ cells. Instead of barcoding every cell in a sample, FIND-seq allows for the isolation of HIV DNA+ cells and is compatible with the intact provirus detection assay (IPDA), which can differentiate many forms of defective provirus. In this talk, I will discuss single-cell analysis technologies, their application in HIV cure research, and the technical advances needed to sequence the replication-competent HIV reservoir.

**Steatotic Liver Disease in Persons Living With HIV**

Jennifer Price
University of California San Francisco, San Francisco, CA, USA

**Background:** Steatotic liver disease (SLD) is a rising cause of liver-related morbidity and mortality worldwide, including among persons with HIV (PWH). This presentation will review the new nomenclature for SLD, which is predominantly metabolic dysfunction-associated (MA SLD) and can overlap with alcohol-related liver injury. We will also review the initial work-up of a patient with SLD and elevated liver enzymes, and we will discuss a stepwise approach to assessing for clinically significant fibrosis using non-invasive tests. Finally, we will review current management of PWH and SLD, including lifestyle modification, adjustment of potentially contributory medications, and pharmacologic interventions.

**Hepatitis Delta: What to Know, What to Do?**

Kathrin van Bremen
University of Bonn, Germany

**Background:** Worldwide, between 12 and 60 million people with chronic hepatitis B (HBV) are estimated to be coinfected with Hepatitis Delta (HDV). HDV infection is caused by a defective RNA virus which is only able to replicate in presence of HBV. Although international guidelines recommend testing of Hepatitis Delta in every person with chronic HBV infection, HDV prevalence data and actual numbers are lacking as the testing coverage is poor. There is a wide variation of geographic HDV infection rates with highest prevalence rates in Eastern and central Europe, the Mediterranean basin as well as in West and Central Africa. Hepatitis Delta infection can be prevented through Hepatitis B vaccination, therefore worldwide coverage of Hepatitis B vaccination is indispensable. Shared transmission pathways for HIV, HBV and HDV result in an increased risk for HDV coinfection in persons with HIV (PWH). Recent European data reported HDV prevalence in PWH between 7% and 15%, with the highest rate in people who inject drugs. Interestingly, transmission risks and patterns may be subject to change, as data from Taiwan recently showed an increased HDV incidence in men who have sex with men (MSM). Moreover, increasing migration may also contribute to changes in HDV prevalence. HDV co-infection is known to cause the most aggressive course of liver disease in PWH leading to significantly more liver cirrhosis, hepatocellular carcinoma and eventually an increased rate of liver-related death. Therefore, wide screening coverage is mandatory to detect cases early. With the 2020 European approval of Bulevirtide, a novel entry-inhibitor blocking the HDV-HBV-specific receptor, a new HDV specific drug is now available for treatment of HDV infection as a daily subcutaneous injection. Treatment with Bulevirtide is recommended in persons with HIV/HBV-/HDV-co-infection with compensated liver disease according to EACS guidelines. Recent data have shown a good decline in HDV-RNA and normalization of liver enzymes in PWH which is in the range of HDV treatment responses in HBV/HDV coinfected subjects without HIV. The optimal duration of treatment however, as well as long-term data are still lacking. In conclusion HDV represents a frequently underdiagnosed critical health issue that needs more attention in order to increase HDV diagnosis rate and enable access to new HDV drugs, thereby improving the unfavorable outcome of HDV in HIV/HBV-coinfection.

**Cirrhosis Management**

Mazen Noureddin
Houston Research Institute, Houston, Texas

**Background:** In our up coming cirrhosis management lecture, we’ll cover the diagnosis and management of cirrhosis, with a focus on HIV patients. We’ll discuss diagnostic methods, including medical history, physical exams, and advanced tests like liver function and imaging studies. For management, we’ll explore lifestyle changes, pharmaceutical options for complications, and the challenges of concurrent HIV and cirrhosis treatment. Our goal is to provide a concise yet comprehensive overview to empower you with the knowledge needed to effectively diagnose and manage cirrhosis, particularly in the context of HIV.
Overview of the Case-Based Workshop on Antiretroviral Therapy
Rajesh T. Gandhi
Massachusetts General Hospital, Boston, MA, USA

Background: In this session, Drs. Claudia Cortes and Rajesh Gandhi will present cases to an expert faculty panel from around the world to highlight cutting-edge issues in the care of people with HIV. Topics will include the management of drug-resistant HIV, treating people who are not able to take oral antiretroviral therapy (ART), managing ART during pregnancy, and ART considerations in the setting of HIV/TB coinfection. This fast-paced and exciting interactive session will illustrate current approaches to managing people with HIV and highlight high-priority areas for future research.

Exploring Strategies to Measure and Understand Users’ Preferences in HIV Prevention and Care
José A. Bauermeister
University of Pennsylvania, Philadelphia, PA, USA

Background: Advances in short- and long-acting pre-exposure prophylaxis (PrEP) and Antiretroviral Therapy (ART) have fueled the need to understand how individuals make trade-offs and competing decisions regarding their preferred PrEP and ART modalities. In this presentation, Dr. Bauermeister provides an overview of the state-of-the-science regarding how HIV researchers have conceptualized and measured users’ preferences in HIV prevention and care studies. Dr. Bauermeister will describe key conceptual and methodological approaches to understanding users’ preferences and choices, including a discussion on the value and trade-offs between Discrete Choice Experiments (DCEs), Conjoint Analysis (CJAs), and Stated Preference Methods. Using several case studies, Dr. Bauermeister will illustrate how these data may help characterize how users make decisions about HIV prevention and care regimens, inform market segmentation strategies to reach diverse types of potential users, and contribute to the development of more effective, user-centered clinical decision aids for PrEP and ART product selection and counseling. Insights into users’ preferences hold significant implications for shaping future HIV prevention and care strategies, ensuring their alignment with user preferences, and ultimately advancing the field towards more tailored and effective interventions.

Hybrid Effectiveness Implementation Studies: Unrecognized Challenges and Emerging Directions
Elvin H. Geng
Washington University in St Louis, St Louis, MO, USA

Background: Progress in the HIV response today depends on more than ever use of rich toolbox of efficacious interventions (e.g., PrEP) with reach, equity, sustainability and quality in the real world. One source for existing gaps between identifying efficacious interventions and their use is the is traditional scientific sequence of first efficacy, then effectiveness and finally implementation trials. Hybrid trial designs - studies that seek to study both implementation as well as effectiveness simultaneously - offer important but incompletely realized opportunities to accelerate translational impact. The talk will cover current nomenclature and classification of hybrid trial types (e.g., “Type I”), highlight current evolution in design and distill key insights that aim to have immediate implications for researchers conducting trials in HIV. In addition, however, I will turn attention to challenges inherent in hybrid designs that seek simultaneous investigation of implementation and clinical outcomes that are to date inadequately addressed. First, current literature does not fully consider when and how different implementation strategies change observed subsequent clinical effects. I offer principles to help guide determination of relative importance of implementation vs. effectiveness outcomes and the plausibility of their heterogeneous effects across settings or populations. Second, hybrid designs have not fully addressed unanticipated “adverse” effects of implementation. The talk will therefore offer a typology of adverse effects from implementation strategies and ways researchers can capture such effects. Third, literature does not fully explore the role of implementation outcomes act as mediators of the effects of strategies on service delivery or clinical outcomes. I draw from modern epidemiological methods in mediation to highlight opportunities and pitfalls for analysis of hybrid trials, including potential utility of sequential randomization. Looking forward, I suggest that the next generation of hybrid designs can be improved by use of a causal or explanatory theory of how implementation strategies work and that use of causal diagrams can help to surface these mechanistic relationships. Throughout, I illustrate methodological principles with substantive issues importance to HIV field, such as long-acting injectable medications, differentiated service delivery models, HIV self-testing and other areas of contemporary importance.

Stopping Clinical Trials Early: When and Why
Sally Hunsberger
National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

Background: Monitoring accumulating data as studies are being performed is important. Data and Safety Monitoring Boards (DSMB) are an independent group of experts, established by the study sponsor, to review accumulating safety and efficacy data. While reviewing interim data, the DSMB may recommend stopping the study for efficacy, futility, harm, or toxicity. A recommendation to stop a study is based on a range of considerations, in this presentation we discuss these considerations. A study is stopped early for efficacy when there is strong evidence that an experimental arm of a study is statistically superior to a comparator arm. In this case, it is important to stop a study so participants and the community can receive the best care as early as possible. Deterrents to stopping a study early include: less data to examine secondary endpoints and subgroups, less safety data/long-term safety data, inconsistent results at end of study due to missing data at the interim analysis. A recommendation to stop a study early for futility can be based on effect size or logistics. Futility can be determined statistically when there is very low probability that there will be a significant result at the end of the study, based on current data and hypothesized future data. Logistical futility could occur if: enrollment is too slow to answer the scientific question while the question is relevant, there is a high drop-out rate making the data difficult to interpret, or a lower-than-expected event rate leads to an underpowered study. Statistical errors are of concern when evaluating interim data for efficacy or futility and appropriate statistical procedures are needed. When monitoring for efficacy, it is important to not inflate the α level (the probability of incorrectly concluding the arms of a study are different). When monitoring for futility, it is important to not inflate the β level (the probability of incorrectly concluding the arms are not different). Statistical methods have been designed to appropriately control these errors when evaluating interim data. Stopping a study early is a difficult decision that must take many considerations into account. When recommending stopping a study, a DSMB must carefully balance benefits to study participants against the collective benefits that would be gained by accumulating additional study.

Modern Vaccinology: A Legacy of HIV Research
Bamey S. Graham
Morehouse School of Medicine, Atlanta, GA, USA

Background: Structure-based vaccine antigen design has been a critical determinant of respiratory syncytial virus and SARS-CoV-2 vaccine success stories. Nucleic acid vaccines and vector-based vaccine delivery have been successfully developed for Ebola and COVID-19. The ability to identify B cell lineages with the capacity for broad neutralization and then target those B cells with novel antigens is being applied to new influenza vaccines. Nanoparticle display is being used for improved influenza, COVID-19, and RSV vaccine designs. Heterologous prime-boost vaccines are now approved for prevention of Ebola. Pseudotyped virus neutralization assays are routinely used to analyze immune responses to high virulence pathogens. Advances in flow cytometry and single cell sequencing have made rapid human monoclonal antibody discovery and repertoire analysis feasible. All of these concepts have common roots in efforts to make an HIV vaccine. Despite 40 years of effort and technological advances we still don’t have an HIV vaccine. There are many reasons for this including: 1) rapid establishment of a reservoir of latently-infected cells and infection of immunoprivileged tissues, 2) multiple ways to evade innate immunity, 3) antigenic diversity of infecting strains, 4) genetic variability and rapid T cell immune escape in infected persons, 5) Env conformational evasion of neutralizing antibodies, 6) Env glycan shield against neutralizing antibodies, 7) immunodominance of antigenic sites on Env with low vulnerability to neutralization, 8) paucity of Env targets on virions, 9) mucosal sites of infection, 10) infection of cells critical for induction of immunity, 11) infection of both lymphoid and antigen-presenting Fc-bearing cells, and 12) potential for infection by virus-infected cells as well as cell-free virions. Solving any of these difficult immunological problems creates a potential solution for other infectious and non-infectious diseases. In that respect, despite a small
probability of success, working toward successful HIV vaccines has been and will continue to be one of the most productive scientific activities of our time.

14 Reflections on Ending Pediatric HIV: Back to Basics, Confront the Unexpected, Challenge Assumptions
Dorothy Mbori-Ngacha
Formerly with United Nations Children’s Fund, New York

Background: Over the past two decades remarkable progress has been made in our efforts to reduce vertical transmission of HIV, thanks to support for and investment in ending AIDS among children. Programmes for preventing the transmission of HIV during pregnancy, birth and breastfeeding have had significant impact and averted an estimated 3.4 million infections in children (aged 0–14 years) since 2000. Nevertheless, with 130 000 (90 000–210 000) new infections occurring among children globally in 2022, we are still off-track towards achieving our global target of eliminating vertical transmission as a public health threat by 2025. Each day in 2022, approximately 740 children became infected with HIV and approximately 274 children died from AIDS-related causes, mostly because of inadequate access to HIV prevention, care, and treatment services. In this lecture I will highlight the programmatic developments – taking scientific innovations to scale in policies and programmes – that have underpinned efforts towards the elimination of mother-to-child transmission of HIV over the past decade. The presentation will draw lessons from our past successes and failures in our PMTCT programs and discuss potential approaches to use in addressing the remaining gaps. Key questions that the presentation will reflect on include: Who are we missing in our response? What do we need to do differently to achieve and sustain universal coverage of PMTCT programs? How can we accelerate progress to achieve our 2025 targets? Finally, potential areas for on-going research will be highlighted.

15 Unveiling the Power of Uganda’s LGBTIQ Advocacy in Shaping HIV Response and Health Care Access
Frank Mugisha
Sexual Minorities Uganda (SMUG), Kampala, Uganda

Background: Embark on an exploration of Uganda’s ongoing battle against state-sponsored homophobia and transphobia, this presentation sheds light on the vital role of LGBTIQ advocacy in shaping the country’s HIV response and healthcare access. In the face of eroding rule of law and political repression, discriminatory laws criminalizing consensual same-sex conduct have created an environment of fear and vulnerability. This has resulted in severe consequences, including family banishment, unemployment, and pervasive discrimination, further exacerbated by limited access to targeted healthcare for LGBTIQ Ugandans. Despite commendable achievements, such as thwarting the Sexual Offences Bill 2019 through tireless advocacy, the recent enactment of the Anti-Homosexuality Act 2023 presents a formidable challenge to healthcare access and health-seeking behavior. This presentation delves into the harsh realities confronted by the sexual and gender-diverse community, navigating a landscape deeply entrenched in religious propaganda and community-driven initiatives for change. Join us on this journey as we explore the intricate interplay of policy, legislation, and the lived experiences of the LGBTIQ community, all while striving for progress in healthcare access amidst the alarming prevalence of HIV and STIs in Uganda.

16 What’s New in HIV Vaccines: Vaccine-Induced Immune Responses
M. Juliana McElrath
Fred Hutchinson Cancer Center, Seattle, WA, USA

Background: Development of an effective HIV vaccine remains an elusive goal. Yet the search has accelerated, driven by the imperative end HIV and new directions based on promising leads in preclinical and phase 1 clinical trials. Recent breakthroughs in understanding how broadly neutralizing antibodies interact with their target epitopes on the HIV envelope spike have led to new HIV envelope immunogen designs that may offer a path to eliciting these types of antibodies. Also, novel vaccine designs for inducing highly functional CD8+ T cells with antiviral activities. By leveraging new vaccine technologies and delivery systems, and tailored trial designs, we aim to speed up the pace of progress.
New developments in mRNA technology, adjuvant technology, and AI-based immunogen design will accelerate developing germline-targeting vaccination approaches that yield bNAbs.

20 Novel Immunization Strategies to Move on Down the Road
Darrell Irvine
Massachusetts Institute of Technology, Cambridge, MA, USA

Background: Progress is being made in the development of HIV Env immunogens with the potential to activate and expand B cell precursors capable of maturing to produce broadly neutralizing antibodies (bNAbs) that could protect against HIV infection. However, such precursors are generally very rare in the human B cell repertoire and must make challenging sets of mutations in their antigen receptors (via somatic hypermutation in germinal centers, GCs) in order to produce bNAbs. In addition, once expanded and matured, these B cells must be driven to differentiate into long-lived plasma cells that can produce high levels of protective circulating antibodies and provide long-lasting protection. These immunological challenges to the development of an HIV vaccine are being tackled by a variety of approaches to formulate engineering vaccine immunogens in a manner that can optimally stimulate the B cell response, amplify GC responses, and promote high titers of output antibody production. This talk will summarize recent advances in the design of nanoparticle immunogens, vaccine dosing schedules that augment the GC response, potent new adjuvants in development, and the use of new vaccine technologies such as mRNA to promote the humoral immune response.

21 Epidemiology of Perinatally Acquired HIV Among Adolescents and Young Adults
Mutsawashe Bwakura-Dangarembizi
University of Zimbabwe, Harare, Zimbabwe

Background: Adolescents (10–19 years) and youth (15–24 years) living with perinatally acquired HIV represent increasing proportions of people living with HIV. Improved access to antiretroviral therapy globally has resulted in children who acquire HIV around the time of birth or through breastfeeding surviving into adolescence. While many are thriving, a significant proportion face several challenges that can affect their long-term outcomes. In particular, poorly controlled HIV disease resulting from suboptimal early regimens and nonadherence, together with the toxicities of some ARV drugs, can predispose them to long-term sequelae including HIV-associated complications and other comorbidities. This talk will be focusing on the state of the epidemic, the global epidemiology and trends of perinatally acquired HIV among adolescents over the years in the different geographical locations. acquired HIV experience poorer HIV-related outcomes compared to younger children and adults with HIV, dying more often and experiencing greater challenges in terms of treatment adherence and staying in care.

22 Historic Evolution of HIV and Mental Well-Being Among Adults Living With Perinatally Acquired HIV
Exzer Kang
Howard University, Washington, DC, USA

Background: Adults living with perinatally acquired HIV (PHIV) in the United States (US) have experienced key historic epochs of an epidemic since the 1990s. They navigated four lifespan periods — childhood, adolescence, young adulthood, and adulthood — spanning over three decades of the HIV epidemic. This has indelibly shaped the contours of their development and mental health. Influenced by a “complex interplay of individual, social and structural stresses and vulnerabilities” (World Health Organization, 2022), mental health is not merely the absence of mental disorder – it is a state of mental well-being. This paper explores three dimensions of mental well-being — emotional, psychological, and social (Westhorp & Keyes, 2010) — among Black adults living with PHIV in the US, with a focus on their formation in concert with two historic markers — treatment innovation and illness stigma. Drawing from a qualitative study of 20 Black adults living with PHIV in New York City and a select review of the history of pediatric HIV in the US, three dialectic themes will be presented and aligned with emotional well-being (Terminal Illness vs. Chronic Condition), psychological well-being (Innocence vs. Vulnerability), and social well-being (Visibility vs. Invisibility). These findings suggest that distinct narratives of the HIV epidemic, and its many iterations, have enduring effects on individual narratives of mental well-being among a welcomed generation of adults living with PHIV.

23 Cardiometabolic Risks and Complications: Adolescents and Young Adults With Perinatally Acquired HIV
Sahera Dirajlal-Fargo
Ann and Robert Lurie Children's Hospital, Chicago, IL, USA

Background: Antiretroviral therapy (ART) scale-up has dramatically reduced rates of pediatric HIV mortality and morbidity. Children living with perinatally acquired HIV (PHIV) are living through adolescence and well into adulthood, such that adolescents now represent the largest growing population living with HIV. This presentation aims to discuss the literature describing the prevalence of cardiometabolic complications and the research gaps that remain, as well as opportunities to optimize research and care. There are continued challenges in determining the risk of cardiometabolic co-morbidities in adolescents and young adults with PHIV, and their risk factors differ compared to adults with horizontally acquired HIV. Data suggest evidence for subclinical cardiometabolic complications in PHIV in the setting of newer ART and include: 1) Cardiovascular: evidence of functional cardiac abnormalities, subclinical vascular disease and endothelial dysfunction; 2) Metabolic: evidence of alterations in adipose tissues, dyslipidemia and insulin resistance. In addition, previous exposure to thymidine analogues continue to cause increase risk of metabolic complications in this population. Novel techniques available techniques in imaging and omics may help identify early cardiometabolic abnormalities in this population as well as mechanistic pathways. Further studies are needed to understand the long term risk and management strategies in adolescents with PHIV to prevent complications to avoid diabetes and cardiovascular disease.

24 Why Can't We Do Better at Diagnosing Syphilis?
Ina Park
University of California San Francisco, San Francisco, CA, USA

Background: This session will utilize challenging case studies to review methods for syphilis diagnosis, including direct detection, serology (non-treponemal and treponemal tests), molecular diagnostics and point of care testing.

25 The Burgeoning Epidemic of Congenital Syphilis
Angelica Espinosa Miranda
Ministry of Health of Brazil, Brasilia, Brazil

Background: Congenital syphilis (CS) is transmitted from an infected mother to her unborn child during pregnancy, leading to severe health complications such as stillbirth, miscarriage, infant death, and maternal and infant morbidity. These adverse outcomes can be prevented through timely screening and treatment during antenatal care. The increasing prevalence of CS is associated with several challenges, requiring a comprehensive approach involving improved healthcare infrastructure, enhanced access to antenatal care, strong testing and screening programs, and extensive education initiatives to mitigate the impact on maternal and child health. Addressing the underdiagnosis of syphilis in pregnancy, especially in regions with limited healthcare access, is essential to avoid missed opportunities for screening and early detection. While reliable and accessible syphilis testing during pregnancy, including treponemal and non-treponemal tests, is essential for early detection, challenges may arise due to limited testing facilities, cultural stigma, and the complexity of implementing comprehensive screening programs. Ensuring that infected pregnant women receive timely and appropriate penicillin treatment is another critical measure to prevent CS, as penicillin is the only effective treatment during pregnancy. However, challenges such as limited healthcare access, potential allergic reactions, and penicillin shortages in some countries must be addressed. The stigma surrounding sexually transmitted infections and societal attitudes can discourage pregnant women from seeking testing, treatment, and follow-up care. Therefore, addressing these sociocultural factors is essential to create an environment where pregnant women feel comfortable accessing healthcare services. Despite specific recommendations from the World Health Organization aligned with sustainable development goals, CS remains a public health concern in many countries, with higher rates in developing countries and emerging cases in developed nations. Educating communities, healthcare providers, and pregnant women about syphilis risks, the importance of antenatal care, and available preventive measures is crucial to increase awareness and enable early intervention in CS cases. Syphilis, despite being an ancient infection, presents ongoing challenges that require strategic approaches to enhance the healthcare network’s capacity and improve the quality of care provided to pregnant women.
26 Syphilis: Management Conundrums
Khali G. Ghanem
The Johns Hopkins University, Baltimore, MD, USA

Background: As the rates of syphilis continue to increase, clinicians are caring for patients with complex clinical presentations, and they are facing challenging management dilemmas. In this session, we will answer the following questions:

- How do we approach rapid plasma reagin (RPR) titers that fail to decline appropriately following therapy? What do RPR titers that increase following treatment mean?
- What is the optimal management of patients with neurosyphilis, ocular, and otic syphilis?
- Are additional doses of benzathine penicillin G necessary in patients with these complications?

28 Accelerating Tuberculosis Elimination: Short-Course Prevention
Vidya Mave

Background: Tuberculosis (TB) is among the leading cause of morbidity and mortality from an infectious disease, among patients with and without HIV, worldwide. Despite cost-free 6-month anti-TB therapy (ATT), the cure rates for TB have been suboptimal due to inadequate exposure/adherence to ATT, resulting in higher risk of failure, relapse, or acquired drug resistance, particularly in the setting of HIV. Recent research developments demonstrated that highly potent ATT regimens can allow shortening of TB treatment for both drug-sensitive and drug-resistant TB in adult, adolescent, and paediatric populations. In addition, shortened TB preventive therapy containing highly potent rifamycins and isoniazid is as good as the traditional 6-9 months of ATT. However, the ATT, causing a higher risk of failure, relapse, or acquired drug resistance. Potent ATT regimens can allow shortening of TB treatment for TB in adolescents, causing a higher risk of failure, relapse, or acquired drug resistance.

31 HIV-1 Genome Packaging During Virion Assembly: Selecting the Right RNA
Wei-Shou Hu
National Cancer Institute, Freden dred, MD, USA

Background: During virus assembly, HIV-1 must identify and selectively package the unspliced viral RNA into nascent virions to transfer genetic information to its progeny. A vast majority of HIV-1 virions contain two copies of full-length unspliced HIV-1 RNA that form a dimer, indicating that the RNA packaging is a regulated and efficient process. The viral polyprotein Gag orchestrates virus assembly and mediates RNA genome packaging. During this process, Gag preferentially binds unpaired guanosines within the highly structured 5' untranslated region (UTR) of HIV-1 RNA. Additionally, the HIV-1 unspliced RNA provides a scaffold that promotes Gag-Gag interactions and virus assembly, thereby ensuring its packaging. However, not all HIV-1 unspliced RNAs are created equal. Recent studies showed that HIV-1 uses neighboring sequences as transcription start sites to generate multiple unspliced RNA species with a few nucleotides difference at the 5' end. However, these 99.9% identical RNAs can differ functionally, and one species of unspliced HIV-1 RNA is preferentially packaged over other nearly identical RNAs. These studies reveal the complex regulation of HIV-1 genome packaging process.

32 Virion Maturation: Folding Into the Right Shape
Mamuka Kvaratskhelia
University of Colorado, Aurora, CO, USA

Background: HIV-1 capsid is a closed conical structure formed during virion maturation. It houses the viral RNA genome and key viral enzymes reverse transcriptase and integrase needed for conversion of the single stranded viral RNA into double stranded DNA and its subsequent integration into a host cell chromosome. The viral capsid consists of the capsid protein (CA) arranged predominantly into hexameric lattices, as well as into 12 pentamers, which introduce curvature at the capsid periphery to completely close the conical structure. The capsid assembly is mediated by the cellular polyanion inositol hexakisphosphate (IP6), which binds to central arginine rings in pentamers and hexamers to stabilize these crucial assembly intermediates. Recent structural studies have elucidated a molecular switch that directs CA assembly into pentamers and hexamers. Lenacapavir (LEN, Gilead Sciences) is the first-in-class capsid targeting, long-acting and highly potent antiretroviral. Mechanistic and structural studies have revealed a multimodal mechanism of action of the inhibitor. LEN inhibits both early and late steps of HIV-1 replication with picomolar concentrations. Yet, the underlying mechanism for such a high potency of LEN is unclear. We have developed a LC-MS/MS based methodology to quantitate LEN concentrations in virions and found that sub-stoichiometric inhibitor to CA ratios hyper-stabilize HIV-1 capsid and block infection. The inhibitor remains stably bound to HIV-1 capsid for >24 h. Furthermore, we have investigated an additional antiviral activity of LEN during virion maturation. Our biochemical assays uncovered that LEN binding to CA monomers specifically interferes with the formation of pentamers, whereas the inhibitor promotes the assembly of hydrophobically interacted hexameric lattices. The ability of LEN to offset the delicate balance between pentamers and hexamers resulted in formation of defective or atypical assemblies of CA both in vitro and in virions. These findings provide a new insight into molecular mechanisms of action of LEN.

33 Intact HIV Capsids facilitate innate immune evasion and enter the nucleus via karyopherin mimicry
Gregory Towers
University College London, London, UK

Background: HIV has a core built of around 250 capsid protein hexamers and exactly 12 pentamers. This cone shaped capsid contains the viral single-stranded RNA genome which is converted into a double stranded DNA genome by encapsidated reverse transcription, catalysed by viral reverse transcriptase. Early data suggested that viral capsids come apart or "uncoat" before viral DNA synthesis, but we now understand that the infectious core are the ones that remain intact until after nucleic entry. Genetic studies have associated conserved capsid features with recruitment of a series of specific host cofactor proteins in the cytoplasm, in nuclear pores and in the nucleus and pioneering microscopy techniques have clearly illustrated intact capsids in the nucleus and associated them with successful infection. We have shown that the single pandemic HIV-1(M) lineage capsid has unique features that promote evasion from innate immune sensors. We hypothesise that this is explained by a more
sophisticated regulation of the timing and location of capsid uncoating and genome release by the cofactors. This more effectively hides viral DNA and therefore permits more effective innate immune evasion. We hypothesise that this in turn promotes human-to-human transmission because innate immune activation is expected to reduce viral replication at the site of exposure and therefore establishment of infection. In collaboration with David Jacques and Till Boecking of the University of New South Wales we have also discovered that, despite their huge size, intact capsids can traverse nuclear pores through mimicking karyopherin nuclear transport proteins. Capsids do this by recruiting the FG motifs found in the nuclear pore diffusion barrier to a conserved binding pocket found in each capsid monomer. Thus, HIV capsids are molecular machines that have evolved to protect the process of viral genome synthesis from innate immune detection and to transport the genome across the cytoplasm, through nuclear pores, and to chromatin where they uncoat, and release genome, in exactly the right location and at exactly the right time for successful, undetected integration into host chromatin.

32 Novel Markers of Hepatitis B: Clinical Utility For New Treatment Strategies
Fabien Zoulim
Institut National de la Santé et de la Recherche Médicale; Université Claude Bernard Lyon 1, Lyon, France

Background: In the context of novel treatment strategies aimed at HBV cure by eliminating or silencing the cccDNA reservoir, its non-invasive evaluation with blood viral biomarkers is critical to monitor intrahepatic viral clearance and guide treatment cessation. One of the caveats of these biomarkers is their ability to accurately distinguish biomarkers expressed from cccDNA versus those expressed from integrated viral sequences. HBsAg can be expressed from viral sequences integrated in the host genome which undermines its value in predicting cccDNA levels and transcriptional activity, particularly in HBsAg+ patients and patients under NUC therapy. Circulating HBV RNA (cir-B RNA) concentration is a promising novel biomarker for antiviral treatment monitoring. Quantification of cir-B RNA, measured with research laboratory developed assays, have good predictive power for both on-treatment serological response and off-treatment durability. New generation investigational assays allowed the quantification of cccDNA derived viral RNAs in serum but not from integrated sequences. Cir-B RNA detection correlates with cccDNA transcriptional activity in NUC treated or untreated patients. HBV core-related antigen (HBcrAg), a composite biomarker of core/pre-core derived proteins, is thought to be mainly expressed from cccDNA derived RNA template and was also shown to have a good predictive value for antiviral treatment response. cir-B RNA and HBcrAg are usually correlated. The combination of undetectable cir-B RNA and HBcrAg at the end of treatment is more predictive for sustained suppression of replication off-treatment compared with either biomarker alone. Other biomarkers are in development to assess the cccDNA reservoir, e.g. the quantification of phosphorylated and non-phosphorylated HBeAg in the blood circulation. These investigational biomarkers are now used for the evaluation of target engagement and antiviral efficacy to assist the development of new antivirals (Capsid Assembly Modulators, SirNA, antisense oligonucleotides, etc.) and immunomodulatory agents (check point inhibitors, TLR agonists, therapeutic vaccines, etc.). Altogether, these non-invasive viral markers show promise for a deep phenotyping of patients and show potential for patient stratification and novel treatment evaluation.

33 Advances in HBV Immunotherapy: The Beginning of the End?
Adam Gehring
University Health Network, Toronto, Canada

Background: Encouraging data shows that new combination therapies are beginning to achieve HBsAg loss in a significant proportion of chronic hepatitis B (CHB) patients. In some patients, HBsAg loss is durable, achieving functional cure, while others relapse, with HBsAg becoming detectable again during follow up. What determines cure vs. relapse remains unclear but, in the absence of a sterilizing cure, the immune system is believed by many to be a critical component to long-term, off-treatment HBV control. Therefore, immunological adjuvants such as IFN-α, therapeutic vaccines, checkpoint inhibitors, and innate immunomodulators are being/will be combined with novel direct acting antivirals (DAAs) to try and increase the durability of cure. Whether immunomodulation will be a requirement for durable cure, or endogenous immunity will be sufficient, is likely to be patient/cohort specific.

This presentation will look at immune correlates of viral control, clinical trials where immunomodulation enhances functional cure rates, and immunological questions that need to be addressed in DAA therapies.

34 How New WHO Guidance Can Transform Hepatitis B in Sub-Saharan Africa
Olufunmilayo Lesi
World Health Organization, Geneva, Switzerland

Background: The presentation will provide the latest epidemiology of HBV in sub-Saharan Africa (including Hepatitis delta) and highlight the unique consideration and status of elimination (and comparison to other regions); recent evidence related to reducing new infection (HB PMTC); strategies for transforming the HBV public health response; and highlights from the updated 2024 WHO guidelines for hepatitis B treatment and care.

35 Introduction to DoxyPEP: Understanding the Issues
Chase Cannon
University of Washington, Seattle, WA, USA

Background: New strategies are needed to address persistently increasing rates of bacterial sexually transmitted infections (STI). Recent trials demonstrate that doxycycline post-exposure prophylaxis (doxy-PEP) significantly reduces the risk of chlamydia, syphilis, and gonorrhea in cisgender men and transgender women who have sex with men. One study found doxy-PEP did not reduce risk for STI in cisgender women, potentially due to low adherence to the intervention in the trial. Despite its potential benefits for STI risk reduction in some populations, several potential implications of doxy-PEP in the near and longer term merit consideration. This presentation will review current evidence to frame the knowns and unknowns about doxy-PEP, highlight the range of doxy-PEP guidance and position statements, discuss the basis for concerns about antimicrobial resistance, and introduce future considerations for implementation to maximize benefit and minimize harms related to doxy-PEP use.

36 DoxyPEP: Should We Worry About Antimicrobial Resistance?
Beatrice Bercot
St Louis Hospital, APHP, Université Paris City, Paris, France

Background: Increased rates of bacterial sexually transmitted infections (STIs) are reported among men who have sex with men (MSM), particularly among those using HIV pre-exposure prophylaxis (PrEP). Interventions to reduce the incidence of STIs are needed. In the field of STI prevention using doxycycline on-demand post-exposure prophylaxis (PEPdoxy), we are witnessing significant advances. Three large-scale randomized clinical trials (ANRS peregay, ANRS Doxyvac trial, DoxyPPE) have demonstrated a reduction of more than two-thirds in the incidence of bacterial STIs among MSM, notably for Chlamydiae trachomatis (CT) and Treponema pallidum (TP) infections, thanks to PEPdoxy. However, efficacy against Neisseria gonorrhoeae (GC) varies according to pathogens responsible for STIs, as already observed for GC, which could worsen, or a new acquisition of resistance for CT and TP which has never been described, and (ii) the impact of doxy-PEP on the composition of the various microbiota and the pathogens present in these flora, in particular for Escherichia coli and Staphylococcus aureus. This presentation will give an overview of the available data on the impact of doxy-PEP strategies on the microbiota and the molecular mechanism conferring resistance. These studies on the microbiome and antimicrobial resistance surveillance for bacterial STIs should be continued over time to fully assess the impact of this strategy.

37 Implementation of DoxyPEP: Challenges and Opportunities
Stephanie E. Cohen
San Francisco Department of Public Health, San Francisco, CA, USA

Background: Doxycycline post-exposure prophylaxis (doxy-PEP) is highly effective in reducing bacterial STIs among men who have sex with men (MSM) and transgender women (TGW). Optimizing the public health impact of this highly effective STI prevention tool while minimizing potential risks will require a multi-pronged, equity-centered implementation strategy. The US Centers for Disease Control and Prevention draft doxy-PEP guidelines give a grade 1A recommendation for doxy-PEP for MSM and TGW with a history of an STI in the past year. Other national, state and local health jurisdictions have released guidance with broader or more limited eligibility for the use of doxy-PEP. Key
implementation considerations include: Who should be offered doxy-PEP and in what settings? What counseling should be provided to individuals receiving doxy-PEP? How can doxy-PEP uptake, adherence and persistence be maximized across age and racial/ethnic groups? What training and tools do providers need to integrate doxy-PEP into their practice? What is the risk of antimicrobial resistance in STI and non-STI pathogens with longer-term use of doxy-PEP? and how should this be monitored? How can the effect of doxy-PEP on STI incidence be monitored and how success can be measured? What are the findings to date in terms of the uptake and impact of doxy-PEP in early adopter cities? In this symposium, we will review these key questions, highlight areas of controversy across existing doxy-PEP guidelines, and discuss the available evidence on doxy-PEP implementation outside of the clinical trial setting.

38 Overview of the Global Displacement Crisis
Mesfin T. Tessema
International Rescue Committee, New York, New York

Background: Globally, over 110 million people have been forcibly displaced from their homes due to various factors such as conflict, violence, persecution, and human rights abuses. Displaced people, especially women and children face a heightened risk of sexual violence, exploitation, and trafficking exposing them to increased health risks including exposure to HIV infection. Many face discrimination and stigma. Addressing HIV in these contexts is not only a protection and human rights issue but also a public health priority.

39 The End of Oral? How Long-Acting Formulations Are Changing the Management of Infectious Diseases
Charles W. Flesner
The Johns Hopkins University, Baltimore, MD, USA

Background: Despite having near-perfect single tablet regimens, adherence to daily oral HIV treatment and prevention is unacceptable low in many settings. Long-acting and extended-release drugs and formulations hold promise for solving this problem and improving outcomes, facilitating the achievement of WHO targets for controlling this epidemic. The first LA/ER formulations for HIV treatment and prevention are now approved and available but are underutilized in LMICs, mainly because of access issues. There is a need for products with less frequent dosing, greater patient convenience, and reduced risk of virologic failure, as well as regimens that also suppress hepatitis B virus infection. Novel products must be accessible in resource-limited settings and for vulnerable populations that include children, adolescents, and pregnant women. Long-acting drug delivery also has the potential to transform the treatment and prevention of other infections including tuberculosis, malaria, and viral hepatitis. This presentation will review recent advances in formulation science that are going to help make available better replacements for daily oral drugs for HIV and many other infectious diseases.

40 Diagnostics 4.0: The Future of Diagnostics for HIV and Related Infections
Nitika P. Pai
McGill University, Montreal, Canada

Background: Overview Since the late 1980s, novel HIV screening and diagnostic technologies have led the way in the field of HIV/STBBI and changed the landscape of diagnostics in Infectious Diseases. Global interest and awareness of timely testing have translated to an enhanced momentum for integrating and efficiency with data-driven decisions. We will discuss these technologies’ role in catalyzing digital health transformation as it unfolds with many solutions. This talk will attempt to provide an overview of exciting technological developments and solutions in HIV/STBBI screening and diagnosis. Solutions that promise efficient surveillance/tracking, education/empowerment, rapid access to testing and treatment, or an offer of personalized care lead to clinical/public health impact. We will also offer a vision of near-term scientific advancement in diagnostics. Learning objectives and outcomes After this session, participants will be able to 1) Identify the state-of-the-art screening and diagnostic technologies for HIV/STBBI 2) Identify the evidence on promising digital and machine learning technologies that will impact health service delivery. 3) Potential for digital health transformation with impact on operational and patient-centered outcomes. 4) Envision the future of diagnostics and tech-enabled digital transformation in HIV/STBBI.

41 From Mechanisms to Therapeutics: Eliminating HIV-infected Cells by the CARD8 Inflammasome
Liang Shan
Washington University in St. Louis, St. Louis, MO, USA

Background: A successful curative strategy for HIV should aim at selective elimination of HIV-infected cells. The ‘shock-and-kill’ approach involves inducing viral gene expression to trigger immune clearance of infected cells. One of the main obstacles is the presence of immune escape variants in HIV reservoirs. Therefore, broadly reactive T cell and antibody responses are required to overcome viral diversity. Another challenge lies in effectively triggering cell death. Immune effector cells including CD8+ T cells and NK cells induce apoptosis in target cells. However, quiescent CD4+ T cells, which are a major reservoir for the virus, are less susceptible to T cell and NK cell attacks compared to cycling T cells. Moreover, cells harboring latent HIV may undergo positive selection to become more resistant to apoptosis. To this end, our goal is to identify new immune pathways that specifically target highly conserved viral components and effectively induce cell death in HIV reservoirs. We reported that caspase recruitment domain-containing protein 8 (CARD8) is an innate immune sensor that can be activated through proteolytic cleavage of its N-terminal fragment. In HIV-infected cells, CARD8 cannot detect the virus because the viral protease remains inactive as a subunit of unprocessed Gag–Pol polyprotein. Some HIV–specific non-nucleoside reverse transcriptase inhibitors (NNRTIs) can trigger intracellular viral protease activation. Treating HIV–infected macrophages and CD4+ T cells with NNRTIs leads to CARD8–mediated caspase 1 activation and pyroptotic cell death. Targeting CARD8 for HIV reservoir elimination offers two significant advantages. Firstly, the viral protease activity against CARD8 is well conserved across major HIV subtypes. Secondly, CARD8 exhibits high functionality in quiescent CD4+ T cells and can trigger cell death independently of apoptosis. Further research should be conducted to explore and discover more potent activators of CARD8 that specifically target HIV-infected cells. This will help in enhancing the effectiveness of CARD8–based therapies for eliminating the HIV reservoir.

42 Mechanisms to Therapeutics: TACK Molecules Kill HIV-infected Cells Through Inflammasome Activation
Tracy L. Diamond
Merrick & Co, Inc, Rahway, NJ, USA

Background: The viral reservoir, consisting of both HIV–1—expressing and latently infected cells, necessitates life-long antiretroviral therapy (ART) to suppress HIV–1 replication in people living with HIV (PLWH). Current ART blocks viral replication and prevents spread to healthy cells. In doing this it maintains, but does not reduce, the HIV infected cell reservoir. A common approach to address the reservoir is known as “shock and kill”, which seeks to reactivate latent HIV–1 such that cells can be targeted and eliminated through viral cytolysis or host cellular immunity. This approach has yielded some clinical success in inducing viral reactivation but has had little to no impact on reducing the reservoir. Cytotoxic agents that are selective for HIV-infected cells could enhance or complement such a strategy. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are common components of ART that have been designed to enzymatically block the viral reverse transcriptase (RT), however studies have demonstrated that some NNRTIs also have a secondary mechanism of action resulting in HIV–specific cell kill. Through this targeted activator of cell kill (TACK) mechanism NNRTIs enhance HIV–1 Gag–Pol dimerization causing premature HIV–1 intracellular protease maturation which induces HIV–1 cytootoxicity through CARD8 inflammasome activation. Although current marketed NNRTIs have potent RT inhibitory activity, they contain weak to no secondary TACK activity and therefore are not expected to be relevant inducers of HIV–cell kill at clinical exposures. Here, we optimized this secondary activity to identify TACK compounds with potencies compatible with clinically achievable concentrations. Pre-clinical proof of concept for cell kill was demonstrated in ex vivo studies with cells from PLWH as well as in an
43 From Transcriptomics to Therapeutics: A Host Restriction Factor That Targets HIV Expression

Rasmi Thomas
Walter Reed Army Institute of Research, Silver Spring, MD, USA

Background: Advances in unbiased next-generation sequencing methods for characterizing all RNA transcripts in a sample have revolutionized scientific research in the past decade, leading to discoveries related to how variation in people impacts responses to disease outcomes, vaccination and therapeutics. Transcriptomics assays express from every gene in an organism’s genome to reveal the global pattern of transcription. Measuring gene expression in different tissues, conditions, or at different times can reveal details of an organism’s biology that result in differential disease outcomes. Transcriptomics was first developed as bulk RNA sequencing (RNA-seq), which yields an aggregate of all gene expression in many cells. Recent single cell RNA-seq (scRNA-seq) techniques provide transcriptomics data for individual cells, which delineate changes in each cell relative to another. This talk describes how we used scRNA-seq to measure not only the host transcripts, but also HIV RNA (vRNA) expression in people living with HIV during acute HIV-1 infection (AHI). This method allowed us to identify host factors restricting HIV-1 transcripts in vivo without any additional laboratory manipulation. A subset of CD4+ T cells with a memory phenotype had the most vRNA+ cells, recapitulating previous data using other methods. Frequency of these cells correlated with important clinical parameters like plasma viremia and cell-associated HIV DNA levels, suggesting that the identified vRNA+ cells were biologically meaningful. When analyzing viral transcripts as a continuous phenotype across individual cells, we identified several host genes for which higher expression levels associated with lower vRNA. PTMA showed the strongest association with lower vRNA expression. This observation was validated in additional participants from different world populations and HIV subtypes. PTMA expression at timepoints after initiation of treatment showed an inverse correlation with frequency of vRNA+ cell measured during AHI, suggesting that changes in PTMA even in the absence of viremia can affect viral transcript levels. In vitro overexpression experiments provided direct evidence that expression of the prothymosin α protein encoded by PTMA inhibited HIV-1 transcription and expression. These results identify prothymosin α as a host factor that restricts HIV-1 infection in vivo, which has implications for viral transmission and cure strategies.

44 From Structure to Therapeutics: CD4 Mimetics “Open” Env and Sensitize HIV-1-Infected Cells to ADCC

Andrés Finzi
Centre de Recherche du CHUM, Université de Montréal, Montreal, Quebec, Canada

Background: Combination antiretroviral therapy (cART) controls human immunodeficiency virus (HIV-1) replication and extends the longevity of persons living with HIV-1 (PLWH). Even with optimal cART, latent HIV-1 proviruses persist in long-lived reservoirs, from which virus rebounds within days to weeks of treatment interruption. Therefore, new approaches aimed at eliminating HIV-1 reservoirs are needed. Persistently infected cells can potentially be eliminated by harnessing host immune responses. One promising strategy relies on the ability of immune effector cells to kill infected cells expressing the HIV-1 envelope glycoprotein (Env). Non-neutralizing antibodies (Abs) targeting highly conserved CD4+-induced (CD4i) Env epitopes have the potential to eliminate infected cells by antibody-dependent cellular cytotoxicity (ADCC). Unfortunately, as a countermeasure, the HIV-1 Vpu and nef proteins downregulate CD4, preventing the exposure of ADCC vulnerable CD4i Env epitopes. Small CD4i-mimetic compounds (CD4mcs) can “open-up” Env, exposing vulnerable CD4i Env epitopes and sensitizing HIV-1-infected cells to ADCC. A cocktail of two families of CD4 Abs (anti-cluster A and anti-coreceptor binding site Abs) together with a CD4mc was shown to significantly decrease the size of the reservoir and delay viral rebound after cART interruption in humanized mice. A summary of the development of new families of CD4mcs with improved potency, new cocktails of CD4i Abs with higher ADCC activity, their impact on the viral reservoir in humanized mice and ongoing studies in non-human primates will be presented. These new developments have the potential to accelerate the application of this powerful approach to PLWH.
The Ring Comes Full Circle: Navigating the Complex Landscape of Biomedical Prevention Post-Phase III

Leila E. Mansoor
Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa

Background: The biomedical prevention landscape enters a pivotal phase as dapivirine (DPV) vaginal ring progresses beyond Phase III clinical trials. This presentation explores: the role of open-label extension (OLE) studies in refining Phase III results, insights from recent PEPFAR-based data in Zimbabwe on real-world ring use, and the intricate balance required in shaping future target product profiles within a complex regulatory landscape. Topical HIV prevention methods offer not only localized defense but also systemic protection. DPV ring Phase III trials revealed a 30% efficacy point estimate, rising to a promising 50% in women over 21. While pivotal for safety data, OLE studies complement and refine Phase III outcomes. Incorporating real-world scenarios, diverse populations, and long-term observations, these studies provide essential insights for optimizing DPV ring deployment. DPV ring OLE studies found increased counterfactual efficacy estimates. Increased adherence and retention relative to the randomized controlled trials were also noted. WHO’s endorsement propels DPV ring implementation in 11 countries, targeting empowerment for young women, who often face heightened vulnerability due to age-related power dynamics, influencing the cycle of transmission. Favorable views expressed by women in Zimbabwe, underscore the tangible impact of DPV ring implementation. Examining usage patterns, adherence challenges, and demographic factors bridges the gap between clinical trials and practical implementation in resource-limited settings. Additionally, local manufacturing in South Africa aims to reduce ring costs significantly. Navigating a complex regulatory environment, DPV ring demands careful consideration for future target product profiles. Despite Phase III outcomes, studies in adolescents (MTN-034/REACH), pregnant women (MTN-042/DELIVER), and breastfeeding women (MTN-043/B-Protected) affirm safety and acceptability. Emerging 90-day variants and multi-purpose prevention technologies (MPT) underscore the pivotal balance for widespread acceptance and global success. “The Ring Comes Full Circle” represents a crucial juncture in biomedical prevention. Through open-label studies, real-world insights, including vulnerable populations, and a nuanced regulatory approach, we pave the way for effective, user-friendly, and globally applicable interventions. Beyond Phase III perils, DPV ring, with evolving iterations, contributes to our global arsenal against HIV.

Challenging the Dogma of Event-Driven PrEP

Jenell Stewart
Hennepin Healthcare and University of Minnesota, Minneapolis, MN, USA

Background: HIV is preventable with the use of daily oral PrEP or long-acting injectable PrEP for all populations regardless of sex or gender. Additionally, event-driven PrEP is an option for cisgender men who desire intermittent use of medications. The IPERGAY Trial provided an important answer, intermittent dosing of PrEP is an effective method to prevent HIV, and as the only randomized controlled trial of event-driven PrEP, we are left with a persistently unanswered question, does event-driven PrEP only work for cisgender men? Real-world data and observational studies in Paris, San Francisco, Bangkok, Johannesburg, Amsterdam, and Harlem suggest that event-driven PrEP is desired, effective, and cost-effective. Cisgender women in Southern and Eastern Africa have reported a preference for an HIV prevention method that did not require taking daily pills, which made event-driven dosing more user-friendly and acceptable. However, efficacy data on event-driven PrEP among cisgender women are lacking. Pharmacokinetic studies on drug levels in plasma, rectum, and genital samples from cisgender women, cisgender men, and transgender women suggest that drug levels are higher in the rectum than the vagina and lower in the presence of estrogen, and yet despite these differences across sex and gender, daily oral Tenofovir Dapivirine-Emtricitabine is highly effective at preventing incident HIV in all populations. Are these differences in drug concentrations a valid justification limiting event-driven PrEP use to cisgender men who have sex with men? In many settings, cisgender women who are not interested in or have barriers to daily dosing, even in settings of infrequent sexual exposures, have been dissuaded from using oral PrEP due to concerns about the need for perfect adherence for vaginal protection. Seven doses of oral PrEP a week offers excellent protection against HIV and recent retrospective data demonstrated that four single strength doses a week had similarly high protection among cisgender women suggesting that dosing frequency needs may not differ by sex assigned at birth.
100 Safety Profile and Immunogenicity of a Phase I Clinical Trial Using Germline-Targeting Trimer GT1.1

Karijn van der Straten,1 Tom Caniels,1 Emma Reiss1, Annelou L. van der Veen,1 Katrina Millard2, David C. Montefiori3, Georgia D. Tomaras1, Dagna Laufer2, Vincent Philpotts4, Michelle J. Klouwen1, Marit van Gils3, Rogier W. Sanders,1 David Diemert1, Godelieve J. de Bree1, Marina Caskey2
1Academic Medical Center, Amsterdam, Netherlands, 2The Rockefeller University, New York, NY, USA, 3Duke University, Durham, NC, USA, 4International AIDS Vaccine Initiative, New York, NY, USA, 5George Washington University, Washington, DC, USA

Background: An effective HIV-vaccine should induce broadly neutralizing antibodies (bNabs) targeting the viral envelope glycoprotein, which is challenged by the low frequencies of bNab precursor B cells. Pre-clinical studies have shown the ability of the BG505 SOSIP.GT1.1 gp140 (GT1.1) vaccine to prime bNab precursor B cells, including those targeting the CD4-binding site. Here, we report the first safety and immunogenicity data from a first-in-human clinical trial using GT1.1.

Methods: This phase I, double-blinded, placebo-controlled, dose-escalating vaccination trial was conducted at two US sites and one in the Netherlands. Participants received intramuscular injections of either 30µg (low-dose) or 300µg (high-dose) of the GT1.1 vaccine with AS01B adjuvant system, or saline placebo at 0, 8, and 24 weeks. Reactogenicities were reported during the 15 days post-vaccinations, Serious AEs for the entire study period. Serum antibody binding and neutralization responses were quantified using BAMa and TZM-bl pseudovirus neutralization assays, respectively.

Results: We enrolled 47 adults without HIV (low-dose: n=20, high-dose: n=19, placebo: n=8), with an average age of 30 years and an similar sex distribution between groups. Ninety-four percent of participants reported at least one solicited Adverse Event (AE). Most AEs were graded mild (59.2%) or moderate (37.7%). There were no significant differences in number of AEs between the vaccine administrations (Chi-Squared test, p=0.17), or dose groups (p=0.13). No vaccine-related Serious AEs were reported. All vaccinated participants developed detectable GT1.1-binding serum antibodies at weeks 10 and 26, with the high-dose recipients showing a higher response rate after the first vaccination (10.5% low-dose vs. 31.5% high-dose) and significantly higher responses at week 10 (p=0.008) (Fig. 1A). GT1.1 neutralizing antibodies (NAb) were more prevalent in the high compared to the low-dose recipients after the second (68% vs. 28%, respectively) and third vaccination (100% vs. 89%, respectively) (Fig. 1B). Serum NAb activity was at least in part directed against the CD4-binding site.

Conclusion: The adjuvanted GT1.1 vaccine has an acceptable safety and reactogenicity profile and induced a potent vaccine-specific serum antibody response. Here, a higher GT1.1 dose induced a more rapid and robust serum antibody binding response without compromising safety. Thus, germline-targeting trimer GT1.1 may represent a promising vaccine candidate for priming bNab responses in humans.

B

GT1.1 pseudovirus neutralization

Figure 1. Serum antibody responses against BG505 SOSIP.GT1.1 gp140 following GT1.1 vaccination. Serum IgG binding (A) and neutralization responses (B). MFI: Median Fluorescent Intensity, ID50 50% inhibitory dilution. Serum responses were compared using a Mann-Whitney U test. P<0.05; *p<0.01; **p<0.001; ***p<0.0001.

101 Vaccine Combining Slow-Delivery and Follicle-Targeting Improve Humoral and Germinal Center Responses

Kristen A. Rodrigues,1 Y. Jason Zhang, Arees Aung, Duncan Morgan, Laura Maorio, Paris Yousefpour, Justin Gregory, Parastoo Amlashli, Maureen Buckley, J. Christopher Love, Darrell Irvine
Massachusetts Institute of Technology, Cambridge, MA, USA

Background: Vaccines generate humoral immunity by activating antigen-specific helper T cells and B cells, which cooperate in germinal centers (GCs) to generate high-affinity antibodies. To provide antibody-based protection, an HIV vaccine will likely need to induce broadly neutralizing antibodies (bNabs). To date, identified bNabs exhibit unusual features like extensive and improbable mutations and lengthy CDR3s; their precursor clones are often rare and have low affinity for HIV Env trimers. Emerging strategies to prime these rare precursors involve achieving prolonged antigen exposure, formulating multivalent and particulate immunogens, or employing potent adjuvants; these strategies have been shown to amplify GCs in preclinical studies, but combining these effects in a single shot is challenging.

Methods: Toward this goal, we engineered aluminum hydroxide (alum), the most common clinical adjuvant, into a slow-delivery vehicle by tagging HIV Env trimers immunogens with short phosphoserine (pSer) linkers to promote alum-binding-individual alum particles are decorated with antigens and mimic virus-like particles. We term this multivalent antigen and alum complex “alum-pSer.” In parallel, we developed a potent saponin-based adjuvant, SMNP, to modulate the inflammation. In this study, we examined the impact of alum-pSer, SMNP, or combining these two adjuvants (Abstract Figure) on humoral response and GC B cells in mice using flow cytometry, ELISA, scRNAseq, and microscopy.

Results: The alum-pSer approach bolstered immunogen bioavailability. The SMNP adjuvant enhanced lymph drainage and immunogen transport to follicles. Notably, the combination exhibited remarkable synergy in amplifying humoral responses compared to SMNP or alum-pSer alone, eliciting 3.3-fold and 56-fold more antigen-specific GC B cells on day 14 and 1.8-fold and 12-fold greater serum IgG titers on day 28, respectively. The combination exhibited remarkable synergy in amplifying humoral responses compared to SMNP or alum-pSer alone, eliciting 3.3-fold and 56-fold more antigen-specific GC B cells on day 14 and 1.8-fold and 12-fold greater serum IgG titers on day 28, respectively. The combination exhibited remarkable synergy in amplifying humoral responses compared to SMNP or alum-pSer alone, eliciting 3.3-fold and 56-fold more antigen-specific GC B cells on day 14 and 1.8-fold and 12-fold greater serum IgG titers on day 28, respectively.

Conclusion: These findings indicate this simple combination adjuvant approach achieves both sustained antigen availability and altered antigen localization, productively steering the GC response in a way conducive to priming rare B cell clones against protective HIV epitopes and broadly applicable to other pathogens.
Multi-Specificity Is a Common Trait of HIV-1 Broad Neutralizing Capacity

Peter Rusert¹, Chloé Pasin², Merle Schanz², Boris Pedenko², Daniel Schmidt¹, Irene A. Abela³, Nikolai Friedrich¹, Cyrille Niklaus¹, Michele Sickmann¹, Jaqueline Weber¹, Gregory Effantin¹, Winfried Weissenhorn², Huldrych F. Günthard³, Roger Koyou², Alexandra Trkola¹
¹University of Zurich, Zurich, Switzerland, ²University Hospital Zurich, Zurich, Switzerland, ³Université Grenoble Alpes, Grenoble, France

Background: Multi-specific responses have been described in rare people who evolved broadly neutralizing antibody (bnAb) activity in HIV-1 infection but their relevance remains unclear. Here we screened the Swiss HIV Cohort Study for multi-specificity by examining the XbnAb cohort comprising bnAb inducers (N=304) identified in the Swiss 4.5k Screen (Rusert Nat Med 2016).

Methods: Plasma neutralization fingerprints of bnAb inducers were evaluated against a 41-virus multiclad panel and compared to reference fingerprints of known bnAbs using an established Spearman based correlation method and a novel delineation strategy, termed virus panel classification, we developed to record multi-specificity. BCR were cloned from 16 cases by 10bGenomics and bnAbs of interest epitope mapped (mutational scanning, competition binding, mutant neutralization, cryo-EM).

Results: Classical bnAb plasma delineation of the XbnAb cohort assigned a single bnAb activity in 90% of plasmas. As the prediction records a dominant activity, secondary multi-specific activity cannot be excluded also in successfully assigned plasmas. This hidden extent of multi-specificity can be substantial as shown by elite neutralizer S51434, a Subtype B infected, slow progressor with predicted silent face activity based on the Spearman correlation method, as shown by elite neutralizer S51434, a Subtype B infected, slow progressor with predicted silent face activity based on the Spearman correlation method.

Conclusion: bnAb inducers, substantiates the wide occurrence of multi-specificity.

CD4 Binding Site Glycan-Deficient SHIVs Elicit Broadly Neutralizing Antibodies in Rhesus Macaques

Daniel J. Morris¹, Hui Li¹, Jimery Lora¹, Kirsten Sowers¹, Christian Martella¹, Yingying Li¹, Barton F. Haynes¹, Tongqing Zhou¹, Peter D. Kwong¹, George M. Shaw¹
¹University of Pennsylvania, Philadelphia, PA, USA, ²Duke Human Vaccine Institute, Durham, NC, USA, ³Institutes of Health, Bethesda, MD, USA

Background: Previous work has demonstrated that modifying soluble HIV-1 envelope (Env) trimers to remove glycans around the CD4 binding site (CD4bs) can immunofocus potent neutralizing antibody (NAb) responses to this epitope. However, such immunogens generally did not elicit broadly neutralizing antibodies (bNAbs). Understanding how to better boost these responses can inform vaccine design. Infection of rhesus macaques with replicating simian-human immunodeficiency viruses (SHIVs) bearing WT glycan-intact EnvS rarely elicits bNAbs targeting the CD4bs. Here, we designed novel SHIVs lacking glycans surrounding the CD4bs (197, 363, and 462) to test the hypothesis that infection with these evolving SHIVs could immunofocus, boost, and affinity-mature CD4bs-targeted NAb and bNAbs responses.

Methods: We disrupted glycosylation sequons at the above residues in three SHIVs bearing primary transmitted/founder EnvS (CSH505, BS505 and CH1012) and used these to intravenously infect a pilot cohort of 14 rhesus macaques (RMs). RMs were monitored to evaluate viral kinetics, Env sequence evolution, and NAb and bNAb development.

Results: 9 of 14 RMs exhibited ideal viral kinetics for further analysis. All 9 RMs developed potent autologous neutralizing responses targeting the protein surface beneath the engineered glycan hole. 4 of 9 RMs developed responses capable of neutralizing heterologous glycan-deficient viral strains. Longitudinal sequencing of plasma viral RNA revealed rapid, sequential restoration of the deleted glycans as well as CD4bs bNAb escape mutations arose temporally with rising neutralizing titers. Two RMs developed antibody responses capable of neutralizing WT heterologous viruses. Based on these results, we downselected from these constructs SHIV.CH505.CD4bs.GH to infect an additional 8 RMs. We observed CD4bs-targeted neutralization breadth in an additional 6 RMs. Epitope mapping of these broad responses showed that 7 of 8 RMs targeted the CD4bs.

Conclusion: These results show that SHIV Env trimers with targeted glycan deletions can immunofocus B cell responses to the CD4bs. Viral evolution in response to these glycan hole targeted NAbS and bNAbs can boost and affinity mature these responses, in some cases leading to bNAbs that target WT heterologous viruses with intact glycan shields. Ongoing studies will isolate and characterize mAbs responsible for this breadth and analyze Env-Ab coevolution to identify key EnvS that can be selected as priming and boosting immunogens.
for single-cell clonal expansion of NHP NK cells, which maintain a conserved NHP NK cell phenotype of CD3- NKG2A/C+ CD16+ CD56+ following expansion. Autologous peptide-loaded BLCL elicited a broad-spectrum of rhNKCL cytotoxic responses, with higher response for SIV ENV-derived peptide.

**Conclusion:** In summary, these findings elucidate antigen-specific NK responses during SIVmac infection dependent on MHC-E-mediated SIV-derived peptide presentation. Also, we introduced a ground-breaking method for the clonal expansion of single NK cells in RM. This study may provide valuable insights into antigen-specific NK cells, including the establishment of epigenetic, metabolic, transcriptional, and phenotypic profiles. Such insights could be leveraged to enhance antiviral NK cell activity in future vaccine strategies and cell therapies.

105 **[18F]F-AraG PET Imaging Reveals Unique Tissue T-Cell Activation Patterns Across HIV Infection States**

**Basic Science:**

Timothy J. Henrich, Robert Flavell, Michael J. Peluso, Kofi Asare, Maya Aslam, Emily Fehrman, Meghann C. Williams, Viva Tai, Rebecca Hoh, Youngho Seo, Jelena Leve, Steven G. Deeks, Henry VanBracklin

1University of California San Francisco, San Francisco, CA, USA, 2CellSight Technologies, San Francisco, CA, USA

**Background:** Non-invasive tools that can test the hypothesis that abnormal T cell activation in various tissues differs across HIV-1 disease states are needed. We used **[18F]F-AraG**, a small-molecule PET tracer highly specific for activated T cells (CD8+CD4+), to compare whole-body T cell activation states in people with HIV (PWH) on and off ART, and experiencing post-intervention control (PIC), compared to uninfected control participants.

**Methods:** **[18F]F-AraG (~5mCi) was administered i.v. to 13 PWH (12 male, one transgender female), 8 on ART, 2 viremic, 3 PIC following combination immunotherapy and 6 uninfected volunteers (3 male, 3 female) followed by whole-body PET-MR imaging. Maximum and mean standardized uptake values (SUVmax/mean) were calculated for regions of interest (ROI) and compared across cohorts using non-parametric tests adjusted for multiple comparisons.

**Results:** We observed significantly higher **[18F]F-AraG** SUVmean and SUVmax in many tissues (nasal turbinates, axial bone marrow, distal spinal cord/ cauda equina, lung parenchyma, pulmonary artery, and rectal wall) in PWH compared to uninfected controls (all P<0.05; Fig 1). Elevated T cell activation was evident even among those on ART (VL<40 c/mL) in these ROI with the exception of lung tissue. Interestingly, tracer uptake was significantly lower in uninfected controls, regardless of disease phenotype. We previously observed and all PWH had lower T cell activation in inguinal lymph nodes compared to uninfected controls, but not in lungs or nasal turbinates. There was a significant difference in tracer uptake in male versus female control participants in any ROI and analyses of all PWH excluding female controls yielded similar results.

**Conclusion:** This study is the first to show persistent T cell activation in bone marrow, pulmonary artery, and naso and gut tissue across a range of HIV-1 disease states using non-invasive PET-MR imaging. Interestingly, T cell activation in lung parenchyma appears to be driven by viremic PWH, and all PWH had lower T cell activation in inguinal lymph nodes compared to uninfected controls, regardless of disease phenotype. We previously observed high uptake of a HIV gp120-specific bnAb PET tracer in inguinal lymph nodes from viremic and ART-suppressed PWH suggesting that combinations of non-invasive imaging tools may play an important role in determining the interplay between host immune responses and viral persistence.

106 **Biomarker Signatures in Phase I/II Study With PD-1 Inhibitor, Budigalimab, in PLWH Undergoing ATI**


1AbbVie, Inc, North Chicago, IL, USA, 2Midway Immunology and Research Center, Fort Pierce, FL, USA, 3McGill University Health Centre Research Institute, Montreal, Canada

**Background:** HIV infection is a major global health problem with ART-free viral control remaining an unmet need. PD-1 blockade offers a promising approach to help address this. We conducted a phase 1b randomized double-blind study (NCT04223804) with an investigational PD-1 inhibitor, budigalimab, in people living with HIV-1 (PLWH) and included planned analytical treatment interruption (ATI) enabling exploratory efficacy and biomarker analyses. Budigalimab was well tolerated, and ART-free viral suppression was observed in a subset of participants. Preliminary exploratory biomarker analyses demonstrating the impact of treatment and ATI are presented.

**Methods:** Safety, pharmacokinetics and pharmacodynamics were examined across multiple intravenous doses of budigalimab (2–10 mg; n=31) and placebo (n=10). Exploratory analyses examined off-ART viral load kinetics, PD-1 receptor saturation, T cell activation and proliferation, plasma cytokines and chemokines, and genome-wide transcriptomic abundances.

**Results:** High peripheral PD-1 receptor saturation was observed for a period of approximately 10 weeks with 10mg Q2Wx4 doses of budigalimab. Six of 9 participants who completed the 10mg Q2Wx4 doses administered during ATI had delayed viral rebound and/or off-ART viral control (HIV-1 RNA <1000 copies/mL), with 2 participants maintaining viral control off ART at <200 copies/mL for over 29 weeks. None of the participants receiving placebo demonstrated this viral load kinetic profile. Increased CD8+ T cell activation was observed and correlated with viral load during ATI. Participants with high viral load during ATI displayed differential transcriptomic trajectories as compared to the profile in participants with low viral load (HIV-1 RNA <1000 copies/mL). Budigalimab treatment was associated with more frequent increases in plasma CXCL9 and CXCL10 during ATI as compared to placebo, though there was no association of these markers with treatment response. A trend in budigalimab-mediated expansion of peripheral CD8+ T cells, T follicular helper-like cells
107 Live Single-Cycle SARS-CoV-2 Vaccine Elicits High Protection and Sterilizing Immunity
Fabian Otto1, David Hauser1, Martin J. Lett1, Jacob Schoen1, Enja T. Kipfer1, Donata Hoffmann1, Nico J. Halwe1, Angele Breithaupt1, Lorenz Ulrich1, Tobias Britzke2, Lorena Urd1, Christian Mittelholzer1, Martin Beer2, Thomas Klimkait1
1University of Basel, Basel, Switzerland, 2University of Oxford, Oxford, United Kingdom

Background: The SARS-CoV-2 pandemic called attention to an urgent need for fast and versatile vaccine development platforms to combat infectious RNA viruses. However, there is a continuous need for new, innovative tools to study viruses with large RNA genomes that have a pathogenic potential for human disease.

Methods: We developed the novel vaccine concept of ‘single-cycle infection viruses’ (SCV) and completed the proof-of-concept for SARS-CoV-2 in the Syrian hamster model. ‘CLEVER’ (Cloning-free and Exchangeable system for Virus Engineering and Rescue), a DNA-based strategy for rapidly generating RNA viruses directly from PCR products, enabled the specific deletion of individual viral genes in a single step. We generated and profiled replication-deficient SARS-CoV-2 variants, for which the missing function could efficiently be complemented in trans. Animals were intranasally prime/boost vaccinated, challenged with wild-type SARS-CoV-2, and assessed for protection against viral infection, transmission, and pathogenesis. Moreover, primary human T cells were utilized to assess the potency in eliciting a specific T-cell response by our SCV candidate.

Results: Several SCV candidates were produced and tested for functionality, stability, and safety (single-cycle properties) in vitro. After intranasal vaccination, animals tolerated the vaccine very well and experienced no weight loss, even when high doses were applied, validating excellent in vivo safety. All animals were fully protected against an autologous challenge with a high dose of infectious SARS-CoV-2 virus. With the specific deletion of three immune-modulatory viral genes sterilizing immunity was achieved, preventing any viral spread to unvaccinated contact animals. Superior immune function was further demonstrated in pre-exposed individuals. The SC vaccine effectively prevented viral infection, transmission, and pathogenesis. Moreover, primary human T cells were utilized to assess the potency in eliciting a specific T-cell response by our SCV candidate.

Conclusion: SCVs can induce broad protection and even sterilizing immunity against viruses, as demonstrated for SARS-CoV-2. We will use the concept to build a vaccine platform targeting other RNA viruses of concern, like Dengue or Chikungunya.

108 Mini-Lecture on Neuropathogenesis of HIV
Sharon R. Lewin
Doherty Institute for Infection and Immunity, Melbourne, Australia

Background: The central nervous system (CNS) presents a unique challenge for HIV cure strategies given the diverse infected cells that persist on antiretroviral therapy (ART) – including infected microglia, astrocytes and circulating T-cells, as well as the presence of the blood brain barrier (BBB), and the adverse acute and chronic consequences of localized inflammation. Numerous studies have now clearly demonstrated the persistence of intact and transcriptionally active cells in the CNS and that the frequency of infected cells and or free virus in the cerebrospinal fluid is associated with adverse neurological outcomes in people with HIV on ART. Using in vitro infection models, latency reversing agents (LRAs) have been shown to have greater potency in astrocytes compared to monocyte derived macrophages. Several clinical trials of LRAs that can cross the BBB, have demonstrated no adverse effects on the CNS in vivo, although the combination of disulfiram with vorinostat had significant neurotoxicity. Newer HIV-specific LRAs utilizing Tat mRNA in a lipid nanoparticles will need to be evaluated in animal models to determine if the theoretical risk of neurotoxicity will be a barrier to further development. With the high interest in immunotherapy and gene therapy currently for an HIV cure, more data is needed to fully understand whether the specific intervention can cross the BBB, whether the intervention is effective in the CNS and whether there are CNS-specific adverse events of concern. Finally, in small prospective studies, antiretroviral therapy interruption has been shown to have limited adverse outcomes on the CNS, but some participants are at higher risk for an adverse outcome and this issue should be considered in the inclusion criteria of future HIV cure clinical trials. Our understanding of the impact of cure interventions on the CNS is critically important but currently limited and needs to be prioritised.

109 Assessing the Contribution of Glial Activation to Cognitive Control and Declarative Memory in PWH
Leah H. Rubin1, Pauline Maki1, Yong Du1, Shannon Eileen Sweeney1, Riley O’Toole1, Raha M. Dartghyeb1, Eran F. Shorer1, Asante Kamkwimala1, Hannah Lee1, Joan Severson1, Il Mi1, Katrina A. Wogalter1, Arnold Bakker2, Martin Pomper1, Jennifer M. Coughlin1
1The Johns Hopkins University, Baltimore, MD, USA, 2University of Illinois at Chicago, Chicago, IL, USA

Background: Virally suppressed people with HIV (VS-PWH) demonstrate impaired cognitive control (CC; executive function) and declarative memory (learning and memory), subdomains of the NIHMH Research Domain Criteria framework. Building on evidence supporting a microglial contribution to central nervous system complications in VS-PWH, we used [11C]DPA-713 with positron emission tomography (PET) to track the translocator protein (TSPO) on activated microglia in the brains of VS-PWH. We hypothesized that VS-PWH (vs. controls) would show higher TSPO in brain regions that subserve CC (lateral prefrontal cortex [lPFC], dorsal anterior cingulate [dACC], inferior parietal lobe [IPL]) and declarative memory (PFC, hippocampus) and that higher TSPO in these regions of interest (ROI) would relate to poorer CC and declarative memory self-report and behavioral measures.

Methods: Twenty-five VS-PWH and 18 demographically-similar control participants completed one 90-min DPA-PET scan with arterial blood sampling, structural brain magnetic resonance imaging, as well as CC (see Figure) and declarative memory measures (Buschke Selective Reminding test, Pattern Separation and Completion, Cognitive Failures Questionnaire-forgetfulness subscale). Regional [11C]DPA-713 total distribution volume (VT) values were estimated using Logan graphical analysis with metabolite-corrected arterial
input function. Regional VT values were compared between serostatus groups (adjusting for TSPO rs6971 genotype) using a linear mixed model with repeated measures. Partial correlations were conducted between ROIs and CC/declarative memory assessments controlling for TSPO genotype.

**Results:** Higher [11C]DPA-713 VT values in CC and DM ROIs were found in VS-PWH vs. controls (P<0.05), with similar magnitude of group difference across the ROIs. In VS-PWH, but not controls, higher [11C]DPA-713 VT in CC regions associated with greater subjective complaints of impulsivity and distractibility (see Figure). Higher VT in CC regions also related to objective measures of CC (Flanker: go/no-go) in each group. For declarative memory, reported forgetfulness but not declarative memory performance associated with higher [11C]DPA-713 VT in PFC in VS-PWH.

**Conclusion:** Higher [11C]DPA-713 VT in CC regions associated with subjective impulsivity and distractibility and lower performance on CC measures. Localized microglial activation in the IFPC, dACC, and IPL may relate to lower CC in VS-PWH. In contrast, associations with declarative memory were not evident.

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**110 Associations Between Depressive Symptom Severity and Incident Stroke Among People With HIV**

Jimmy Ma, Robin M. Nance, David Tirschwell, Stephanie A. Ruderman, Lydia N. Drumhilt, Maile Karris, Lyndsey S. Mixson, Joseph Zunt, Felicia C. Chow, Barbara M. Grippenhaver, Emily Ho, Richard D. Moore, Joseph A. Delaney, Heidi M. Crane, for the Center for AIDS Research Network of Integrated Clinical Systems

Among people with HIV (PWH), depression is a common risk factor for cardiovascular disease (CVD). The relationship between depression and objectively measured cognitive function in PWH is not well understood. In this study, we examined the relationship between depressive symptom severity and incident stroke in PWH, using longitudinal data from the HIV Disease Progression Study (HIVDPS), a multisite prospective cohort study of adults with HIV in clinical care.

**Methods:**
- **Participants:** We included 1,381 PWH who were enrolled in the HIVDPS and had at least two PHQ-9 assessments during the follow-up period (2010-2016).
- **Depressive Symptoms:** Depressive symptoms were assessed using the Patient Health Questionnaire-9 (PHQ-9). Incident stroke was defined as a first-ever stroke event that occurred during the follow-up period.
- **Cognitive Assessment:** Cognitive function was measured using the Center for Epidemiologic Studies-Depression Scale (CES-D) and the Mini-Mental State Examination (MMSE).
- **Statistical Analysis:** The association between depressive symptom severity and incident stroke was examined using Cox proportional hazards regression. Adjustments were made for potential confounders, including age, sex, race, education, smoking status, and baseline antidepressant use.

**Results:**
- Higher depressive symptom severity was associated with an increased risk of incident stroke (HR 1.16, 95% CI 1.01-1.33, P=0.02).
- The association remained significant after adjustment for baseline antidepressant use (HR 1.16, 95% CI 1.00-1.33, P=0.04).

**Conclusion:** These findings suggest that higher depressive symptom severity is a risk factor for incident stroke in PWH. Future studies are needed to explore the mechanisms underlying this association and to evaluate the potential for interventions to reduce stroke risk in this population.

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**111 Carotid Inflammation on FDG-PET is Associated With Lower Cognitive Function in Treated HIV Infection**

Meg Wilson, Shady Abohashem, Ahmed A. Tawakol, Priscilla Y. Hsu, Felicia C. Chow

University of California San Francisco, San Francisco, CA, USA; Massachusetts General Hospital, Boston, MA, USA

**Background:** Studies investigating the relationship between cardiovascular disease (CVD) and cognition in people with HIV (PWH) have largely focused on CVD risk factors and cerebral small vessel disease. We examined the relationship between subclinical carotid arterial inflammation on [18F]-fluorodeoxyglucose (FDG)-PET and cognitive function in PWH.

**Methods:**
- **Participants:** We included 47 PWH (mean age 60, 98% men) with undetectable viral load who underwent [18F]-FDG-PET at moderate to high CVD risk.
- **PET Imaging:** [18F]-FDG-PET was performed using a PET/CT scanner. Carotid inflammation was assessed using standardized uptake values (SUV) in the carotid arteries.
- **Cognitive Assessment:** Cognitive function was assessed using a battery of neuropsychological tests (Hopkins Verbal Learning Test-Revised, Digit Symbol, Grooved Pegboard, Trail Making Test Parts A & B, Stroop, Letter Fluency) and stress measures within 2 weeks of the PET scan.
- **Statistical Analysis:** Carotid inflammation was associated with lower cognitive function in PWH.

**Results:** Adjusting for baseline antiretroviral therapy and CVD risk factors, carotid inflammation was negatively correlated with cognitive function (r=-0.32, P=0.037), even after adjusting for CVD risk (r=-0.34, P=0.029).

**Conclusion:** Carotid inflammation is associated with lower cognitive function in treated HIV infection.
Low Levels of HIV-1 in CSF During ART Are Associated With Neurocognitive Impairment and Inflammation


University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, Yale University, New Haven, CT, USA, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden, National Institutes of Health, Frederick, MD, USA, University of California, San Francisco, San Francisco, CA, USA

Background: Antiretroviral therapy (ART) typically reduces HIV-1 RNA to below the limit of detection of standard vial load (VL) assays and facilitates immune reconstitution, but neurocognitive impairment (NCI) persists in many people on ART. Previous studies have suggested that higher HIV-1 cell-associated DNA and cell-free RNA levels in cerebrospinal fluid (CSF) are positively associated with neurocognitive impairment (NCI), but the mechanisms driving this association are unknown.

Methods: In a cross-sectional cohort of participants (N=78) who were on ART for at least a year and lacked overt neurologic symptoms, we examined whether the amount of HIV-1 RNA in cerebrospinal fluid (CSF) during ART is associated with elevated inflammation and/or NCI. We measured neurocognition by an 11-test battery and also measured plasma and CSF VLs (by both standard [Abbott Real Time assay, limit of detection (LOD) 40 cps/ml] and single copy assays [SCA, HMMcGag assay, LOD 0.25 cps/ml]), cell counts, and 12 inflammatory biomarkers.

Results: ART regimens were NRTI- (34%), PI- (33%), and INStI-based (29%). Median blood CD4+ T cell counts, nadir blood CD4+ T cell counts, and CSF WBC counts were 506 cells/μl, 127 cells/μl, and 1 cell/μl, respectively. The cohort was divided into three groups with increasing HIV-1 RNA levels in CSF (median, IQR cp HIV-1 RNA/ml): (1) undetectable by all assays, (2) detectable by SCA only (0.33, 0.27-1.2 by SCA), (3) detectable by standard assay (40, 40-40 by standard assay). Using Kendall’s tau rank correlation (Figure 1), we observed that levels of HIV-1 RNA in CSF were positively correlated with CSF MMP9 (p=0.001), CSF protein (p=0.001), plasma TIMP1 (p=0.031) and blood CD4+ T cell count (p=0.003) and negatively correlated with total neurocognition z score (p=0.026) and speed of processing z score (p=0.011).

Conclusion: We observed that during ART small increases in HIV-1 RNA that do not reach the level of treatment failure or CSF escape are associated with increased immune activation (CSF MMP9, plasma TIMP1), blood-brain barrier disruption (CSF protein) and neurocognitive impairment (total z score and speed of processing z). This contributes to the growing evidence that persistent exposure to HIV-1 in the CNS during ART is associated with NCI and suggests that inflammation may play an important role in this process.

HIV Transcription Persists in the Brain of People With HIV and Viral Suppression

Janna Jamal Eddine, Thomas A. Angelovich, Jingling Zhou, Sarah J. Byrnes, Carolin Tumpach, Nadia Saraya, Emily Chalmers, Stephanie Marinas, Paul R. Gorry, Jacob D. Estes, Bruce J. Brew, Sharon R. Lewin, Susahma Telwatte, Michael Roche, Melissa J. Churchill

Background: HIV persistence in the brain is a barrier to cure, and potentially contributes to HIV-associated neurocognitive disorders (HAND) that affect ~30% of people with HIV (PWH) despite viral suppression with antiretroviral therapy (ART). Persistent HIV transcription and blocks to transcription have been identified in latently infected CD4+ T cells from blood and lymphoid tissues. However, whether HIV transcription persists in the brain despite viral suppression with ART and is subject to the same blocks to transcription seen in other tissues and blood cells, is unclear.

Methods: HIV transcriptional profiling of autopsy frontal cortex brain tissue from virally suppressed (n=12; undetectable plasma viral load [pVL]: <50 c/mL; 335 CD4+ T cells/mm³) and non-virally suppressed PWH (n=13; pVL: 61,223 c/mL; 8 CD4+ T cells/mm³) was performed using nanowell digital PCR based assays. Associations between levels of HIV transcripts, clinical parameters, and levels of the intact and defective HIV reservoir in frontal cortex tissue as measured by IPDA were assessed by correlative analysis.

Results: Frontal cortex tissue from PWH had HIV TAR (n=25/25) and Long-LTR (n=23/25) transcripts, indicative of transcriptional initiation and early elongation, respectively. Completion of HIV transcription (PolyA) and multiple splicing (Tat/Rev) was evident in frontal cortex tissue from 7/13 non-virally suppressed PWH and from 4/12 virally suppressed PWH. HIV p24 protein was also detected in all PWH with PolyA and Tat/Rev transcripts (11/11), demonstrating production of viral proteins in these individuals. Proximal and distal blocks to transcription were present in both groups. However, the block to proximal elongation (TAR–Long-LTR) was more extensive in virally suppressed PWH than in non-virally suppressed individuals (P<0.05; ratio: 2.7-fold greater block). Levels of all HIV transcripts correlated with levels of total and intact HIV proviruses (P<0.05 for all), demonstrating that the level of HIV transcription is associated with HIV reservoir size in the brain.

Conclusion: These findings demonstrate that the brain is a transcriptionally active HIV reservoir in virally suppressed PWH which may contribute to ongoing neuroinflammation and HAND.

Infected T-Cell Clones Are Shared Across CSF and Blood Compartments in PWH

Meng Wang, Jennifer Yoon, Hailey Reisert, Bibhuprasad Das, Jennifer Chiarella, John W. Mellors, Alina P. Pang, Joshua C. Cytok, Margaret Fikrig, Elias K. Halvas, Yuval Kluger, Serena Spudich, Michael J. Corley, Shelli Farhadian

Yale University, New Haven, CT, USA, University of Pittsburgh, Pittsburgh, PA, USA, Weill Cornell Medicine, New York, NY, USA

Background: The central nervous system (CNS) is a site of persistently infected cells during HIV infection. However, the dynamics of CNS infection and T cell trafficking in PWH are incompletely understood. Here, we utilized single-cell T cell receptor (TCR) and transcriptome profiling of paired CSF and blood from
PWH to gain insights into the dynamics of HIV-1 RNA-producing T cells in both compartments, and under the pressure of ART.

**Methods:** We enrolled eight PWH; seven were on suppressive ART and one had chronic HIV profiled before and 3, 7, and 9 months after ART. We also enrolled six HIV-uninfected controls, demographically matched to PWH. We profiled single cell TCR and RNA from paired CSF and blood using 5’VDJ 10x Genomics scRNA-seq and scTCR-seq. To identify whether there were detectable transcriptionally active HIV-1 RNA-producing cells in CSF and blood, we aligned the single cell transcriptome sequencing reads against consensus and autologous HIV-1 genomes.

**Results:** In total, we examined the single-cell transcriptomes of 129,544 CSF cells and 262,818 PBMCs from PWH and controls. We detected transcriptionally active HIV-1 RNA-producing cells in 8/11 (72.7 %) CSF samples and 6/11 (54.5 %) blood samples, with a higher frequency of infected single CD4+ T cells in CSF than in blood. Among infected CD4+ T cells, a majority (83.6 %) were identified as CD4+ central memory T cells. Differential expression analyses revealed infected CSF T cells displayed a unique transcriptional profile compared to uninfected CSF T cells. We utilized scTCR data to identify 36 T cell clones containing infected cells. Most (78%) of these T cell clones were tissue specific (found in blood or CSF but not both), but some (22%) clones contained infected cells were found in both CSF and blood. Most infected cells belonged to singletons (unique TCRs), but 28% belonged to TCR clones with evidence of clonal expansion. Longitudinally following one PWH before and at three time points after initiating ART, we found infected T cell clones that persisted after ART initiation, in both CSF and blood, including a T cell clone that expanded in the CSF several months after ART initiation.

**Conclusion:** By tracking T cell clones across times and tissue, we find that T cell clones persist in the CNS over time. Infected, identical, and expanded T cell clones are found across tissue compartments. Our findings suggest that maintenance and expansion of infected T cell clones contributes to the CNS reservoir in PWH on ART.

**Antiviral Activity, Safety, and Pharmacokinetics of GS-1720: A Novel Weekly Oral INSTI**

**Carl J. Fichtenbaum1, Mergze Berhe2, Jose Bordoni3, Jacob P. Lalezari4, Godson Oguchi5, Gary Sinclair6, Furong Wang7, Brie Falkard8, Hayyoung Zhang8, Eva Martensson9, Jared Baeten10, Moti Ramgopal10**

1University of Cincinnati, Cincinnati, OH, USA; 2North Texas Infectious Diseases Consultants, Dallas, TX, USA; 3Washington Health Institute, Washington, DC, USA; 4Quest Clinical Research, San Francisco, CA, USA; 5Midland Florida Infectious Disease Specialists, Orange City, FL, USA; 6From Health North Texas, Dallas, TX, USA; 7Gilead Sciences, Inc, Foster City, CA, USA; 8MedImmuno Pharmacology and Research Center, Fort Pierce, FL, USA

**Background:** Significant medical need exists for antiretroviral agents that can be administered less frequently. GS-1720 is an orally bioavailable integrase strand transfer inhibitor (INSTI) with potent antiviral activity and physichemical properties well-suited for a long-acting formulation. We are investigating the antiviral activity, safety, and pharmacokinetics of (PK) of GS-1720.

**Methods:** An open-label, multi-cohort Phase 1b study is being conducted in participants with HIV who are treatment-naive or viremic and off antiretroviral therapy for at least 12 weeks. Based on safety and PK data from a Phase 1a study in healthy volunteers, participants are being administered GS-1720 on Day 1 and Day 2 and followed for a total of 10 days. The primary endpoint is plasma HIV-1 RNA (log10 copies/mL) change from baseline to Day 11. Secondary endpoints include plasma HIV-1 RNA change at Day 8 in addition to PK parameters and safety assessments. Genotypic and phenotypic sensitivity testing to drugs from the INSTI class is also being conducted from samples collected during screening and Day 11 visits.

**Results:** Preliminary PK from the Phase 1a study showed a median half-life of 9.4 days with a single GS-1720 dose of 450 mg. In the first Phase 1b cohort (n=7; 6 males, 1 female and mean age 35) dosed daily on Day 1 and Day 2 with 450 mg, GS-1720 demonstrated an HIV-1 RNA mean log10 copies/mL reduction at Day 11 of 2.44 (95% confidence interval [CI] 2.04, 2.83) and at Day 8 of 2.04 (95% CI 1.72, 2.36). No participants experienced any serious adverse events (SAEs), Grade 3 or higher treatment-emergent AEs, or AEs related to study drug. No treatment-emergent INSTI resistance was observed.

**Conclusion:** GS-1720 demonstrated potent antiviral activity and PK supportive of once weekly oral dosing while being well-tolerated. The observed >2 log10 copies/mL decline in HIV-1 RNA and half-life >1 week in this cohort demonstrates the potential of GS-1720 as part of an oral weekly INSTI-based regimen.
117 VH3810109 (N6LS) in Adults With HIV-1 Who Are ART-Naive: Phase Ia BANNER Efficacy Data
Peter Leone, Alejandro Ferro, Sergio Lupo, Joseph McGowan, Paul Benson, Marisa Sanchez, Stefan Schneider, Paul Wannamaker, Beta Win, Judah Abberbock, Viviana Wilches, Margaret Gartland, Max Latallade, Jan Losos

VII Healthc, Durham, NC, USA; Centro de Investigaciones Medicas, Mar del Plata, Argentina; Instituto Gavi, Rosario, Argentina; "Northwell Health, New York, NY, USA; "Be Well Medical Center, Berkeley, MI, USA; "Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; "Long Beach Education and Research Consultants, Long Beach, CA, USA; "GlassSmithKleine, Bentford, United Kingdom; "GlassSmithKleine, Collegeville, PA, USA; "VII Healthc, Branford, CT, USA

Background: The broadly neutralizing antibody (bNAb) VH3810109 (N6LS), a CD4-binding site antibody with broad and potent neutralizing activity in vitro, demonstrated robust antiviral effect (median viral load (VL) decline of 1.72 log10 c/mL and maximum viral nadir from baseline (BL) of −2.60 log10 c/mL) when given at 40 mg/kg intravenously (IV) in people with HIV-1 in part 1 of the proof-of-concept BANNER study and a good safety profile in parts 1 and 2. We report efficacy data for N6LS administered IV or subcutaneously (SC) in parts 1 and 2 of BANNER.

Methods: BANNER is a randomized, open-label, 2-part, multicenter study assessing safety, pharmacokinetics, and antiviral activity of N6LS in treatment-naive adults with VL ≥ 5000 c/mL. N6LS was evaluated during monotherapy after a single IV infusion or SC injection, followed by 48 weeks of standard-of-care antiretroviral therapy. Monotherapy duration was determined by virologic non-response (VL <0.5 log10 by Day 11) or rebound (VL ≥1.0 log10 over nadir or <0.5 log10 from BL).

Results: Of 62 participants, 8 received 40 mg/kg IV and 6 received 280 mg IV in part 1, 16 received 700 mg IV, 16 received 70 mg IV, and 16 received 700 mg SC in part 2. Most participants were male (94%), White (61%), and Hispanic (82%). Median (range) age was 29 (18-61) years. At BL, median VL ranged from 82%. Median (range) age was 29 (18-61) years. At BL, median VL ranged from 4.1 to 4.5 log10 c/mL across dose groups. Median (range) maximum VL decline ranged from −0.43 (−1.29 to −0.72; 10 mg IV group) log10 c/mL to −1.72 (−2.60 to −0.60; 40 mg/kg IV group) log10 c/mL and was reached in a median of 9 days (Figure). Among responders, median (range) time to rebound ranged from 13 (10-22; 70 mg IV group) days to 35 (12-78; 40 mg/kg IV group) days. Across dose groups, there was a weak-to-moderate correlation between BL viral sensitivity and maximum VL decline. N6LS was well tolerated when given IV or SC, with few drug-related adverse events (AEs) and no serious AEs.

Conclusion: Robust antiviral activity was observed after IV and SC administration of N6LS; response was correlated with N6LS exposure. Response with SC vs IV dosing was lower and likely due to differences in BL susceptibility, serum antibody levels, and slower time to reach C50. Overall, N6LS led to dose-dependent declines in VL consistent with antiviral activity reported for other bNAbs. Results support the ongoing development of N6LS into phase IIb.

118 A First-in-Human Study of the Trispecific HIV-1 Broadly Neutralizing Antibody, SAR441236
Atie Tisbiri, Yu E. Zheng, Edmund Capparelli, Katherine Rodriguez, Randall Tressler, Antoine Deslandes, Katherine Shin, Philip Marzinik, Lucio Gama, Baiba Berzins, Chantal Wimbish, Chih-Jen Wei, Gary Nabel, Daniel R. Kurtzkes, Pablo Tebas

Background: SAR441236 was designed to combine the HIV-1 specificities of VRC01 (CD4 binding site) and PGDM1400 (V1/V2 glycan binding), and 10E8v4 (membrane proximal external region) into one molecule with amino acid modifications (LS) in the Fc-region for half-life extension. SAR441236 provided complete protection against macaque SHIV challenge but has not been tested in people with HIV (PWH).

Methods: ACTG AS377 was a phase I study evaluating the safety, pharmacokinetics (PK), and antiviral activity of SAR441236, a trispecific HIV-1 bNAb. Escalating intravenous (IV) or subcutaneous (SC) single doses of SAR441236 (from 0.3 – 10 mg/kg) or 4 doses at 30 mg/kg IV (every 12 weeks with 72 weeks of follow up) were assessed in ART-treated PWH with plasma viral RNA ≤50 copies/mL. Dose cohorts were randomized 2:1 to SAR441236 or placebo. Single-open label IV doses of 1 mg/kg or 30 mg/kg were assessed in viremic participants (ART-naive or no ART in the preceding 3 months). Primary outcomes were study treatment-related Grade ≥3 adverse events, AUC0-12wk of SAR441236, and the day 7 change in plasma HIV-1 RNA levels in viremic participants.

Results: A total of 52 participants were enrolled and 51 received study treatment. Median age was 53 years; 10% were female, 37% were Black, and 12% were Hispanic. No events met the primary safety outcome (95% CI 0-0.09). Population PK analysis demonstrated an overall clearance (CL) of 137 ± 86 mL/d, a volume of distribution (V) of 63 ± 2.4 L, population SC bioavailability of 35 ± 7%, and a half-life (t1/2) of 38 ± 10 days. SAR441236 CL was 38% higher in viremic cohorts. For the 30 mg/kg single dose in aviremic participants, the AUC0-12wk was 22,292 ± 889 pg·d/mL (mean ± SD). Monte Carlo simulations predicted 30 mg/kg IV every 12 weeks to result in a median steady-state trough of 74 μg/mL (90% PI 25-185 μg/mL). Only 7 of 24 planned viremic participants were enrolled. The mean change to day 7 plasma HIV-1 RNA levels in the 1 mg/kg cohort (n = 5) was −0.10 log10 copies/mL and −0.38 log10 copies/mL in the 30 mg/kg cohort (n = 2).

Conclusion: SAR441236 administration was safe and well tolerated. The PK of SAR441236 was similar to traditional HIV-1 IgG antibodies with half-life extension modifications, such as VRC07-523LS. The favorable PK and convenience of administering a single biologic with three binding specificities support the evaluation of novel trispecific or multispecific bNAbs for HIV-1 treatment or prevention.

119 Safety and Efficacy of VRC07-523LS Plus Long-Acting Cabotegravir in the Phase II ACTG AS357 Trial
Babarafi Taiwo, Yu E. Zheng, Katherine Rodriguez, Leah Burke, Jackie Reeves, Paul Wannamaker, Lucio Gama, Christos Petropoulos, Kimberly K. Scarl, Pablo Beluancham-Zamudio, Ronald D Amico, Katharine J. Bar, Pablo Tebas, for the ACTG AS357 Team

Background: Antiretroviral therapy (ART) is a burden for individuals in treatment, and the development of long-acting ART as a single injection every 2 months could simplify HIV care, possibly leading to improved adherence. VRC07-523LS, a trispecific bNAb that targets HIV-1 CD4 binding site and CCR5, is currently under development as a long-acting injectable regimen (LA-CAB) for the maintenance of virologic suppression in ART-naive adults.

Methods: Participants are adults with HIV, virally suppressed for >2 years, a current CD4 count ≥350 cells/mm3, and susceptibility to VRC07-523LS (IC50, 0.25 pg/mL and a Maximum Percent Inhibition (MPI) >98% on the Monogram PhenoSense mAb Assay using screening PBMCs). In Step 1, participants received 4 or 5 weeks of oral CAB and two NRIs. Those still suppressed entered Step 2 and received intravenous VRC07-523LS (40 mg/kg) Q8 weeks plus intramuscular
LA CAB (600mg load followed by 400mg Q4 weeks). At the end of 48 weeks on Step 2 or premature treatment discontinuation, participants returned to a standard of care regimen for 48 weeks in Step 3. The primary outcomes were: 1) grade 3 adverse event (AE) or treatment discontinuation related to VRC07-523LS and LA CAB; and 2) virologic failure (VF) defined as confirmed viral load (VL) ≥200 c/mL at or prior to week 44 of Step 2. Efficacy analyses were as-treated; participants with VL<200 c/mL at the treatment discontinuation were censored.

**Results:** Analysis included complete Step 1/2 follow-up for 74 participants: 26% cis-female; 51% White (non-Hispanic), median age 54. At baseline, 96% had VL <50 c/mL, median CD4 count 720 cells/mm³. Median IC₅₀ was 0.076 μg/mL, median MPI 99.9%. Seventy-one (96%) participants initiated Step 2 treatment: 61 (86%) completed Step 2 treatment and 10 (14%) prematurely discontinued the regimen (5 VFs, 1 death, and 4 participant/physician request). Twelve (16.9%) participants meet the primary safety endpoint: 11 (15%) had grade ≥3 AEs (mostly chills, myalgia, fatigue) and one discontinued therapy due to a grade 1 infusion-related reaction. The only death was unrelated to study treatment. The five VFs (Table) include two of the three participants with VL ≥500 c/mL at week 4. The cumulative probability of VF at or prior to week 44 of Step 2 treatment was 7.3% (95% CI 3.2-16.0%). The integrase R263K mutation was seen in a participant at VF. Pharmacokinetics and anti-idiotype antibodies results are expected in late 2023.

**Conclusion:** The parenteral maintenance ART regimen of VRC07-523LS plus LA CAB was safe. Most participants maintained viral suppression. Observed potential vulnerabilities should inform future bNAb based ART strategies.

### Table: Characteristics of the five participants with virologic failure on VRC07-523LS plus LA CAB (Step 2)

<table>
<thead>
<tr>
<th>Participant</th>
<th>IC0, μg/mL</th>
<th>CD4, cells/μL</th>
<th>Baseline VL, c/mL</th>
<th>Baseline CD4, cells/μL</th>
<th>Baseline IC50, μg/mL</th>
<th>Baseline MPI</th>
<th>HIV-1 RNA level, copies/mL</th>
<th>VF</th>
<th>Initiation of Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>51351459</td>
<td>&lt;0.01</td>
<td>761</td>
<td>69</td>
<td>511</td>
<td>0.030</td>
<td>94.6</td>
<td>208</td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>40040376</td>
<td>&lt;0.01</td>
<td>432</td>
<td>46</td>
<td>205</td>
<td>0.077</td>
<td>94.3</td>
<td>320</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>35030074</td>
<td>&gt;0.01</td>
<td>627</td>
<td>124</td>
<td>277</td>
<td>0.009</td>
<td>94.0</td>
<td>560</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>31004649</td>
<td>&gt;0.01</td>
<td>613</td>
<td>25</td>
<td>205</td>
<td>0.077</td>
<td>94.3</td>
<td>320</td>
<td>4</td>
<td>Yes</td>
</tr>
<tr>
<td>54004247</td>
<td>&gt;0.01</td>
<td>324</td>
<td>110</td>
<td>20</td>
<td>0.077</td>
<td>94.3</td>
<td>320</td>
<td>5</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Results:** Eleven participants were randomized & treated (Group 1, n=5; Group 2, n=6). Age range was 28–63 years; 3/11 were female; 4/11 were Black; & median CD4 count was 916 cells/μL. There was 1 serious adverse event of soft tissue infection not related to study treatment. No adverse events led to study drug. Safety outcomes were similar between groups. One participant restarted baseline ART due to a protocol violation (chronic hepatitis B infection) & was excluded from the efficacy analysis. At Week 26, 8/10 participants maintained V5 (Group 1: 2/4; Group 2, 6/6). Of the two participants in Group 1 who had virologic rebound, one had sensitivity to TAB & was diagnosed with acute COVID-19 at the time of rebound, & one had sensitivity to ZAB & rebounded at Week 26; both had HIV RNA <100 copies/mL.

**Conclusion:** The long-acting combination of LEN+TAB+ZAB was well tolerated, with a favorable safety profile. All participants in the higher ZAB dose group maintained V5 for 6 months, which suggests that more inclusive sensitivity criteria may be appropriate for treatment studies of LEN+TAB+ZAB when higher bNAb levels are maintained.

### 121 Therapeutic Efficacy of a Triple Combination of HIV-1 Broadly Neutralizing Antibodies

**Boris D. Juerg**1, Victoria E. Walker-Sperring1, Kshitiq Wagb1, Kathryn Stephenson1, Jinyn Liz1, Malika A. Boudries1, Roberto C. Andino1, Lucio Gama1, Elena Giorgi1, Richard A. Koup2, Michael S. Seaman1, Charlotte-Paige M. Rolle1, Edwin DeJesus1, Bette Korber1, Dan H. Barouch1

1Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, 2Beth Israel Deaconess Medical Center, Boston, MA, USA, 3Los Alamos National Laboratory, Los Alamos, NM, USA, 4University of Texas at Houston, Houston, TX, USA, 5National Institute of Allergy and Infectious Diseases, Washington, DC, USA, 6Fred Hutchinson Cancer Center, Seattle, WA, USA, 7National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 8Orlando Immunology Center, Orlando, FL, USA

**Background:** Human immunodeficiency virus type 1 (HIV-1) specifically broadly neutralizing monoclonal antibodies (bNAb) have to date shown limited therapeutic efficacy when administered as monotherapy or as a cocktail of two antibodies. A combination of three bNAb provides improved neutralization coverage of global viruses. Here we show that a triple bNAb cocktail targeting three distinct epitopes on HIV-1 Env results in long-term virologic control in persons living with HIV-1 (PLWH) following discontinuation of antiretroviral therapy (ART).

**Methods:** We first evaluated the pharmacokinetics of the bNAb PGT121, PGDM400, and VRC07-523LS, which target the V3 glycan supersite, V2 apex, and C4 binding site, respectively. We then assessed the therapeutic efficacy of up to six monthly infusions of this triple bNAb cocktail in 12 PLWH who discontinued ART after the first antibody infusion (NCT03721510). Participants were not screened for bNAb sensitivity at baseline.

**Results:** 83% of participants (10 of 12) maintained virologic suppression for the duration of the antibody dosing period for at least 28 weeks. Moreover, 42% of participants (5 of 12) demonstrated virologic suppression for the duration of follow-up for at least 38-44 weeks, despite the decline of serum bNAb concentrations to low or undetectable levels. Early viral rebound in 2 individuals correlated with baseline resistance to PGT121 and PGDM400, whereas late viral rebound in 5 participants in the context of declining bNAb levels was characterized by both sensitive and resistant rebound virus.

**Conclusion:** Our data demonstrate the potential of a triple HIV-1 bNAb cocktail to provide long-term virologic control in the majority of PLWH in the absence of ART. Long-acting versions of these three bNAb are currently being developed for HIV-1 prevention and therapy.

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### 122 Randomized Trial of Cabotegravir and Rilpivirine Long-Acting in Africa (CARES): Week 48 Results

**Cissy M. Kityo1, Ivan K. Mambule1, Simiso Sokhela2, Reena Shah3, Caroline Otiol1, Joseph Musaza4, Kimton Opiyo4, Fiona Cresswell5, Charity Wambui6, Gilbert Ategeka1, Josphat Kosgei7, Logashvari Naidoo8, Fafa A. Boatteng9, Nicholas Paton9**

1Joint Clinical Research Centre, Kampala, Uganda, 2University of the Witwatersrand, Johannesburg, South Africa, 3Aga Khan University, Nairobi, Kenya, 4Infectious Diseases Institute, Kampala, Uganda, 5London School of Hygiene & Tropical Medicine, London, United Kingdom, 6Moi University, Eldoret, Kenya, 7Walter Reed Project—for Kenza, Kenza, Kenya, 8South African Medical Research Council, Durban, South Africa, 9Johnson & Johnson, Accra, Ghana

**Background:** Long-acting injectable therapy (LAT) is a recommended option for individualized treatment of human immunodeficiency virus type 1 (HIV-1) infection in resource-rich settings. Additional evidence is required to
determine the role of LA for treatment programs in Africa, where demographic factors, viral subtypes, prior treatment exposure, prevalence of pre-existing antiviral drug resistance and standardized approach to treatment delivery and monitoring differ.

**Methods:** This ongoing phase 3b randomized, multicentre, open-label trial evaluates efficacy, safety, and tolerability of switching from oral antiretroviral therapy (ART) to LA. HIV 1-positive adults, stable on first-line ART (TDF + FTC/ FTC+EFV/NVP/DTG) with VL <50 copies/ml at screening were enrolled at 8 African sites. Main exclusion criteria were past virologic failure, pregnancy and HIV infection. Participants were randomized (1:1) to continue oral ART (OT group) or switch to cabotegravir (CAB) and rilpivirine (RPV) intramuscular injections every 8 weeks (LA group). VL was monitored every 24 weeks. Primary outcome was the proportion of participants with VL <50 copies/ml at week 48, by FDA snapshot algorithm (non-inferiority margin 10%). Confirmed virologic failure (CVF; secondary outcome) was defined as 2 consecutive VL ≥200 copies/ml. Resistance testing was done retrospectively on archived DNA at baseline in all participants, and at CVF.

**Results:** 512 participants were enrolled (median age 42y; 58% female; 92% on DTG-based ART; 74% with prior NNRTI exposure; 14% baseline archived RPV resistance mutations; 57% viral subtype A1; 21% baseline BMI ≥30kg/m²). Four withdrew by week 48 (2 LA, 2 OT group). At 48 weeks, 248/255 (97.3%) in LA and 252/257 (98.1%) in OT group had VL <50 copies/ml (difference -0.8%; 95%CI -3.4 to 1.8%); demonstrating non-inferiority (Table). One participant in LA group met the definition of CVF. Adverse events of grade ≥3 severity occurred in 24 (9%) in LA and 10 (4%) in OT group; only one adverse event in LA led to treatment discontinuation (injection-site abscess). Treatment satisfaction increased after switching to long-acting therapy.

**Conclusion:** At 48 weeks, CAB and RPV LA showed non-inferior efficacy to standard oral ART when used in the public health approach with sparse VL monitoring and where pre-existing RPV resistance, subtype A1 virus and obesity are common. CVF and acquired resistance was rare. LA was effective and safe and may be considered for use in treatment programs in sub-Saharan Africa.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>LA group (n=256)</th>
<th>OT group (n=257)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥50 copies/mL</td>
<td>248 (97.3)</td>
<td>252 (98.1)</td>
<td>-0.8 (-3.4 to 1.8)</td>
</tr>
<tr>
<td>≥1000 copies/mL</td>
<td>5 (1.9)</td>
<td>3 (1.2)</td>
<td>0.7 (-1.4 to 3.0)</td>
</tr>
</tbody>
</table>

**Table: main virological outcomes by randomised group**

* Mutations to rilpivirine (V108I, V118I and V121V) and cabotegravir (RTICV, N155H and Y143H) at failure. One additional LA participant had VL ≥200 copies/ml at week 48 that could not be confirmed (participant died before repeat); mutations to rilpivirine and cabotegravir were present.

**123 Phase I Safety, Tolerability and Pharmacokinetics of Tenofovir Alafenamide Implants in African Women**

Tanuja N. Gengiah, Quarraisha Abdool Karim, Lara Lewis, Ishana Harkoo, Leila E. Mansoor, Johana Khan, Zainab Khara, Nqobile Myeni, Natasha Samunders, Marc M. Baun, John A. Moss, Catherine Hankins, Bruno Pozzetto, James F. Rooney, Salim S. Abdool Karim

1Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, 2Oak Crest Institute of Science, Monowara, CA, USA, 3Amsterdam Institute for Global Health and Development, Amsterdam, Netherlands, 4Centre International de Recherche en Infectiologie, Saint-Etienne, France, 5Ghoshal Sciences, Inc, Foster City, CA, USA

**Background:** Long-acting antiretroviral formulations offer an innovative solution to support adherence in African women seeking HIV pre-exposure prophylaxis. Micro-tabletted tenofovir alafenamide (TAF), a potent antiretroviral drug with established systemic safety, was formulated in a silicone elastomer sub-dermal implant and tested for safety, tolerability, and pharmacokinetics (PK).

**Methods:** Healthy, adult South African women at low HIV risk enrolled in the CAPRISA 018 Phase I Trial. To assess initial safety, six women received a single TAFRA (110mg) implant with an estimated 0.25mg/day in vivo release rate for 4 weeks (Group 1). Thereafter, 30 women randomized 1:1 to either one or two TAF implants, assigned blinded in a 4:1 active (n=24) to placebo (n=6) ratio, for 48 weeks (Group 2). Implant safety (systemic and implant site reactions (ISRs); frequency, severity grading, time to resolution), tolerability (scheduled vs early removals), and PK evaluations (TAF plasma concentrations, and active metabolite tenofovir diphosphate [TFV- DP] in peripheral blood mononuclear cells (PBMCs) with a target protection threshold set at 36 fmol/10⁶ cells) were assessed throughout implant placement and 4 weeks post-removal.

**Results:** Study participant median age was 26 years and 13% had a history of contraceptive implant use. Common ISRs (Figure 1) were mostly mild (Grade 1) with scarring, hyperpigmentation, and induration, continuing for the duration of the study. In Group 2, Grade 3 ISRs, reported in two participants, resolved on implant removal. Early implant removal prior to 48 weeks occurred in 11 (37%) (10 active arm) in a median (IQR) of 19 (10-27) weeks post-insertion, mostly due to ISRs. Plasma TAF was detectable in 77% of samples from Group 2 participants in the active arm, with 100% and 50% detection 0.5 and 6 hours post insertion while median (IQR) TFV-DP concentrations were 3.9 (1.7-13.3) and 14.8 (6.0-29.1) fmol/million cells in women with 1 and 2 active implants respectively, with 15% of samples reaching or exceeding the target concentration.

**Conclusion:** In this first-in-human TAF implant trial, we found an expected safety profile, predominantly as insertion site reactions, but sub-optimal tolerability. Implant drug release rates did not reach targeted PBMCTFV-DP concentrations in most participants. Inserting additional implants or increasing release rates would need to be counter-balanced with the potential for increased side effects and reduced tolerability. Additional PK analyses are underway.

**Figure 1:** Duration of insertion related ISRs (Group 2)

Jean-Michel Molina, Béatrice Berçot, Lambert Assoumou, Michele Algarte-Génin, Emma Rubenstein, Gilles Piaïoux, Christine Katlama, Laure Burgers, Cecile Bebear, Nicolas Dupin, Jean-Paul Viard, Juliette Pavie, Claudine Duvivier, Jade Ghosni, Dominique Costagliola

1University of Paris Cit, Paris, France, 2Sorbonne Université, Paris, France, 3University of Bordeaux, Bordeaux, France

**Background:** Interim results of the ANRS Doxyvac trial (NCT04597424) have shown significant reductions in the incidence of chlamydia, syphilis and gonorrhea with doxycycline PEP and a significant reduction in the incidence of a first episode of gonorrhea with the 4CMenB vaccine, but not of cumulated gonorrhea episodes.

**Methods:** MSM on PrEP with a history of STI, were randomized in an open-label factorial design trial to receive doxycycline PEP (200 mg within 24h of condomless sex) or no PEP (2:1); and 2 shots of the 4CMenB vaccine or no vaccine (1:1). Participants were tested centrally at baseline, every 3 months and when symptomatic for N. gonorrhoeae (GC) and C. trachomatis (CT) by PCR in throat, anus and urine with serologic tests for syphilis. The co-primary endpoints were: the incidence of first episode of CT or syphilis from baseline for Doxy PEP and the incidence of a first episode of GC from 3 months for the vaccine intervention, using an intent-to-treat analysis. We used Cox proportional hazard models to compare incidence between Doxy PEP and no PEP adjusted for vaccine intervention and vice versa. Following external evidence, a single interim analysis occurred in September 2022 at the request of the DSMB who recommended to stop the trial for efficacy. All participants were then invited to come back for a final visit which occurred up to February 28, 2023. Final results are presented.

**Results:** Between January 19, 2021, and September 19, 2022, 556 MSM were randomized and 545 were analyzed. Median age: 40 years (IQR 34-48), median of 10 sexual partners in past 3 months. Median follow-up: 14 months. There was no interaction between the two prevention strategies for the primary
endpoints. The incidence of a first episode of CT or syphilis was 8.8 per 100 PY in the Doxy PEP and no PEP arms, respectively (aHR: 0.17; 95% CI: 0.12-0.26). The incidence of a first episode of GC was 45.5 and 68.4 per 100 PY in the Doxy PEP and no PEP arms, respectively (aHR: 0.67; 95% CI: 0.52-0.87). The incidence of a first episode of GC was 58.3 and 77.1 per 100 PY in the 4CMenB vaccine and no vaccine arms, respectively (aHR: 0.78; 95% CI: 0.60-1.01). The incidence of cumulative episodes was 52.6 and 62.4 per 100 PY, respectively (aHR: 0.84 (0.67-1.07). One drug-related SAE was reported (Erythema).

Conclusion: Among MSM on PrEP, doxy-PEP significantly reduced the incidence of CT and syphilis and to a lesser extent of GC. 4CMenB vaccine no longer showed a significant impact on the incidence of GC.

125 Sustained Reduction of Bacterial STIs During the DoxyPEP Study
Open-Label Extension
Annie Luukemeyer,
Deborah Donnell,
Stephanie E. Cohen,
Julia C. Dombrowski,
Cole Grabow,
Claire E. Brown,
Jason A. Cannon,
Erik Vittinghoff,
Hyman M. Scott,
Edwin Charlebois,
Susan P. Buchbinder,
Diane V. Havlin,
Olusegun Soge,
Connie L. Culum,
for the DoxyPEP Study Team

University of California San Francisco, San Francisco, CA, USA;
Fred Hutchinson Cancer Center, Seattle, WA, USA;
San Francisco Department of Public Health, San Francisco, CA, USA;
University of Washington, Seattle, WA, USA

Background: After early demonstration of 65% efficacy in reducing bacterial STIs in the DoxyPEP Study, participants (pts) in the standard of care (SOC) arm were offered doxy-PEP. We subsequently examined uptake, adherence, sexual activity, and incident bacterial STIs, as awareness of STI prevention efficacy may impact patterns of doxy-PEP use and sexual behavior.

Methods: DoxyPEP is an open-label trial conducted in Seattle and San Francisco among men who have sex with men (MSM) and transwomen (TW) living with HIV (PWH) or on PrEP who had a bacterial STI in the past year, randomized 2:1 to doxy-PEP or SOC. After the efficacy threshold was reached in a planned interim analysis, SOC pts were offered doxy-PEP and both arms were followed in an open label extension (OLE) for up to 12 months total. OLE quarters were defined as those with doxy-PEP for a full quarter after 5/2022 results were disclosed. Disclosed self-reported sexual behavior and quarters with ≥1 STI endpoint are compared descriptively during OLE doxy-PEP vs. SOC as-randomized (SOC-AR) without doxy-PEP.

Results: Of the 637 pts enrolled, 279 contributed to OLE follow-up: 193 from the doxy-PEP arm (D-OLE) and 86 of 87 pts from the SOC arm (SOC-OLE). Of those in the OLE: 96% MSM, 4% TW, 78% were on PrEP, 22% PWH, 62% White, 4% Black, 13% Asian, 22% other; 27% Hispanic. ≥1 STI endpoints were observed among PWH and SOC-OLE ppts reported higher median sex partners per quarter: 15 (IQR 5-30) compared to 8 (IQR 5-25) during SOC-AR. D-OLE ppts reported a median of 12 (IQR 5-25) partners per quarter vs. 10 (IQR 4-25) during SOC-AR.

Conclusion: In the OLE period after doxy-PEP efficacy was known, almost all SOC pts accepted doxy-PEP and both OLE groups reported high doxy-PEP coverage (>70%) of condomless sex in the context of a higher number of sexual partners during OLE. Doxy-PEP was associated with sustained decreased rates of incident STIs in both groups during the OLE compared to no doxy-PEP use among pts initially randomized to the SOC arm.

Table: STI endpoints, sex quarters, and doxy-PEP use among DoxyPEP study participants in the SOC as-randomized (SOC-AR) and doxy-PEP groups.

Doxy-PEP SOC-PEP

Table: STI endpoints, sex quarters, and doxy-PEP use among DoxyPEP study participants in the SOC as-randomized (SOC-AR) and doxy-PEP groups.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>SOC-AR (n=637)</th>
<th>SOC-PEP (n=87)</th>
<th>SOC-AR (n=637)</th>
<th>SOC-PEP (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT incidence (%)</td>
<td>11.3 (9.1-16.1)</td>
<td>12.4 (10.5-14.3)</td>
<td>8.8 (7.4-10.3)</td>
<td>10.2 (8.5-11.9)</td>
</tr>
<tr>
<td>GC incidence (%)</td>
<td>45.5 (40.0-51.6)</td>
<td>68.4 (62.9-74.0)</td>
<td>52.6 (46.9-58.3)</td>
<td>62.4 (56.7-68.1)</td>
</tr>
<tr>
<td>Syphilis incidence (%)</td>
<td>4.7 (3.2-6.7)</td>
<td>6.2 (5.3-7.1)</td>
<td>3.6 (2.8-4.5)</td>
<td>4.2 (3.4-5.0)</td>
</tr>
</tbody>
</table>

Significant differences are bolded.

126 Doxycycline PEP: High Uptake and Significant Decline in STIs After Clinical Implementation
Hyman Scott,
Jorge Roman,
Matthew A. Spinelli,
Jason Benza,
Thiago S. Torres,
Susan P. Buchbinder
1San Francisco Department of Public Health, San Francisco, CA, USA;
2San Francisco AIDS Foundation, San Francisco, CA, USA;
University of California San Francisco, San Francisco, CA, USA;
3Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil

Background: Doxycycline as bacterial sexually transmitted (STI) post-exposure prophylaxis (DPEP) has shown high prevention efficacy in clinical trials. We evaluated the uptake and impact of DPEP on Chlamydia (CT), Gonorrhea (GC), and Syphilis incidence among PrEP users in a sexual health clinic in San Francisco.

Methods: DPEP was offered to all active PrEP clients at their clinical visits starting on 11/30/22. We included PrEP clients with at least one STI test who received DPEP for at least 30 days (DPEP users), or never initiated DPEP (non-DPEP users). The ‘pre-DPEP’ period was defined as 6/1/22-11/30/22; and the ‘post-DPEP’ period started after DPEP initiation for DPEP users. STI testing included GC and CT (urine, rectal, and pharyngeal), and syphilis. Adjusted STI incidence rate ratios (IRR) per quarter for the pre- and post-DPEP periods were evaluated, and a controlled interrupted time series (CITS) analysis with mixed-effects Poisson regression used to evaluate intervention effects.

Results: Of the 3,081 active PrEP clients, 1,209 (39%) received DPEP during the study period. Those who received DPEP were racially/ethnically diverse with 33% White, 26% Latinx, 16% Asian, and 4% Black; and the majority were cisgender men (90%), gay (91%), and 30-49 years (62%). The demographics of non-DPEP users were similar to DPEP users. Among DPEP users, any STI incidence declined from 18.1% in the first quarter of the study period to 7.5% in the last quarter. Among non-DPEP users, any STI incidence was stable between the first and last quarter: 7.0% and 6.5%, respectively. In the pre- post-DPEP analysis, DPEP was associated with decreased STI incidence for any STI (IRR: 0.42, 95% Confidence Interval (CI): 0.24-0.74; p=0.003), CT (IRR: 0.33, 95% CI: 0.23-0.46 p<0.001), and syphilis (IRR: 0.22, 95% CI: 0.17-0.54; p=0.001); but not GC (IRR: 0.89, 95% CI: 0.69-1.15; p=0.383). In the CITS analysis DPEP was associated with a significant decline in the slope for any STI incidence among PrEP clients (IRR: 0.67, 95% CI: 0.46-0.96; p=0.03) (Figure 1). This decline was also significant for CT (p=0.021) and GC (p=0.003), but not syphilis (p=0.360).

Conclusion: DPEP uptake was high reflecting strong demand when offered as part of routine PrEP care. Overall STI incidence declined rapidly after implementation demonstrating high impact of this intervention in a real-world setting. Continued evaluation of uptake, adherence, and impact on bacterial STIs will be essential as DPEP implementation expands.
Doxy-PEP Associated With Declines in Chlamydia and Syphilis in MSM and Trans Women in San Francisco

Madeline Sankaran1, David V. Glidden2, Robert P. Kohn3, Courtney Lieb4, Thiago S. Torres4, Susan P. Buchbinder5, Annie Luetteke6, Monica Gandhi7, Diane Havlir8, Janet Q. Nguyen9, Hyman Scott10, Jorge Roman11, Oliver Bacon12, Trang Q. Nguyen1, Stephanie E. Cohen1

1San Francisco Department of Public Health, San Francisco, CA, USA, 2University of California San Francisco, San Francisco, CA, USA, 3Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil, 4San Francisco AIDS Foundation, San Francisco, CA, USA

Background: In October 2022, the San Francisco (SF) Department of Public Health disseminated guidelines through community and public health networks recommending doxycycline post-exposure prophylaxis (doxy-PEP) for men who have sex with men (MSM) and transgender women (TGW) with a history of sexually transmitted infections (STIs) or multiple sex partners. Doxy-PEP’s effect on population-level incidence of STIs is unknown.

Methods: To monitor doxy-PEP uptake at sentinel sites, we tracked the quarterly number of new patients initiating doxy-PEP from three high-volume SF sexual health clinics. To assess the ecological association between doxy-PEP program implementation and citywide STI incidence, we conducted interrupted time series analyses on monthly reported STI cases of chlamydia (CT), gonorrhea (GC), and early syphilis (ES), among MSM/TGW before (7/1/21−10/31/22) and after (11/1/22−11/30/23) release of doxy-PEP guidelines and used autoregressive integrated moving average (ARIMA) models to forecast expected post-period monthly case counts in the absence of doxy-PEP. Observed case counts were based on citywide surveillance data. Analyses were repeated for monthly CT case counts among cis women for comparison.

Results: From 11/1/22 to 9/30/23, 3,288 MSM/TGW initiated doxy-PEP at the three sentinel clinics. Citywidewide, the number of monthly reported CT cases (6.7% of all cases, p<0.0001) and ES cases decreased significantly after the release of doxy-PEP guidelines compared to model forecasts (Figure). By the end of the 13-month post-period, CT cases decreased 51% (95% CI: 39%-60%) and 50% (95% CI: 38%-59%), respectively, compared to expected counts in November 2023. No significant change in GC cases was seen (p=0.087). Among cis women, the number of monthly reported CT cases in the post-period increased significantly (2.45% of all cases, p<0.01).

Conclusion: Release of SF doxy-PEP guidelines and early implementation at high-volume clinics were associated with a substantial sustained decrease in reported SF cases of CT and ES, but not GC, among MSM/TGW over a 13-month period. Other factors, including changes in screening and sexual practices (e.g., in response to mpox), may have contributed to observed trends. Future analyses are planned with extended post-period data to determine whether observed trends continue to align with citywide doxy-PEP uptake and STI incidence.

Figure. Observed and modelled chlamydia and early syphilis cases among MSM and TGW in San Francisco pre and post doxy-PEP implementation

Site-Based HIV Testing Assay Performance for Cabotegravir and TDF-FTC PrEP Failure in HPTN 083

Raphael J. Landovitz1, Emily Voldal2, Brett Hanscom3, Susan H. Eshleman3, Estelle Piwowar-Manning4, Philip Sullivan5, Marybeth McCauley6, Lydia Soto-Torres1, James F. Rooney7, Alex R. Rinehart8, Myron S. Cohen9, Mina Hosseinipour1, Sinead Delany-Morette1, Beatrix Grinsztejn1

1University of California Los Angeles, Los Angeles, CA, USA, 2Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 3The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4FHI 360, Lusaka, Zambia, 5National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA, 6Research Sciences, Inc, Foster City, CA, USA, 7ViiV Healthcare, Brentford, United Kingdom, 8University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 9University of the Witwatersrand, Johannesburg, South Africa, 10Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil

Background: HPTN 083 demonstrated superiority for long-acting injectable cabotegravir (CAB) compared to daily oral TDF-FTC for HIV pre-exposure prophylaxis (PreP) in cisgender men and transgender women who have sex with men (MSM, TGW). The study was conducted at 43 sites in North and South America, Asia and Africa. During the blinded and first unblinded year study periods, site-based HIV testing algorithms included a US FDA cleared rapid test (RT) with results prior to product administration, and a laboratory-based antigen/antibody assay (Ag/Ab) that was not resulted until after product administration. We present the PPV of these tests in people receiving CAB or TDF/FTC PreP.

Methods: All sites performed RTs and Ag/Ab tests at all study visits and required a non-detected HIV RNA within 14d of study entry; some sites performed two rapid tests prior to product administration based on local practice. HIV status was determined by an external, blinded adjudication committee based on site HIV testing and retrospective HIV testing at a central laboratory. Positive predictive value (PPV, 95% confidence intervals [CI]) for initial site-based reactive testing was assessed for permutations of site test results.

Results: Of 4566 enrolled participants, 70 were excluded (results could not be adjudicated, reactive test results at enrollment, or no HIV testing data after enrollment), 48 had a false reactive test, 130 had a true reactive test, and 4322 had no reactive tests. The analysis included data from 113,316 visits, including 177 initial reactive visits with a reactive RT or Ag/Ab test. PPVs for one or two reactive test results (regardless of RT result) were 95% and 83% respectively. The PPVs of one reactive Ag/Ab test (regardless of RT result) are in the Table.

Conclusion: The PPV of one reactive RT plus one reactive Ag/Ab was 100% for both CAB and TDF-FTC. The PPVs of two reactive RTs for TDF-FTC PrEP and CAB PrEP were 95% and 83% respectively. The PPVs of one reactive Ag/Ab with a negative RT performed were low for both groups and were lower for CAB. In the absence of more sensitive testing, a reactive RT plus a reactive Ag/Ab test, or two reactive RTs had sufficient PPV to warrant initiation of ART. In settings where RNA testing is unavailable or infeasible, algorithms using RTs and Ag/Ab tests had high PPV in the context of MSM/TGW PrEP. Lower PPVs of all tests in CAB cases are attributable to lower HIV incidence in CAB arm participants.
129 Safety and Pharmacokinetics of MK-8527, a Novel nRTTI, in Adults Without HIV

Gillian Gillespie1, Russ P. Carters,1 Xiaowei Zang,2 Ryan Vargo,1 Yash Kapoor,1 Arinjita Bhattacharaya1, Jean-Francois Beneff,1 Tom Raynolds,2 Frederic Vanhoupte,2 Sylvie Rottey,2 Randolph P. Matthews,2 Susan Aubrey,3 Stochi,4 Marian Iwamoto5

Merck & Co, Inc, Wayne, PA, USA, 650 Belgium, Brussels, Brussels, Belgium, 5G5 Belgium BV, Antwerp, Belgium, 5G5 Belgium BV, Antwerp, Belgium, 5G5 Belgium BV, Antwerp, Belgium, 5G5 Belgium BV, Antwerp, Belgium

Background: MK-8527 is an oral nucleoside reverse transcriptase translocation inhibitor (nRTTI) in clinical development. Two phase 1 trials evaluated ascending single doses (trial A) and ascending multiple doses (trial B) of MK-8527 in adults (aged 18–55 years) without HIV.

Methods: In trial A, male participants received single oral doses of MK-8527 (0.5–200 mg; fasted) or placebo; 25 mg was also assessed after a high-fat meal. In trial B, male and female participants received 3 once-weekly (QW) oral doses of MK-8527 (up to 40 mg) or placebo. In both trials, participants were randomized (3:1) to receive MK-8527 or placebo. Safety and pharmacokinetics of MK-8527 (plasma) and MK-8527-triphosphate (TP; measured in peripheral blood mononuclear cells (PBMCs)), the active form of MK-8527, were assessed. Results: In both trials, MK-8527 was generally well tolerated. In trial A, adverse events (AEs) were reported in 27 of 34 participants (79.4%); 5 (14.7%) were considered drug-related AEs. In trial B, AEs were reported in 29 of 32 participants (90.6%); 11 (34.4%) were considered drug-related AEs. In both trials, all drug-related AEs were mild or moderate, and there were no serious AEs, events of clinical interest, or deaths. After single doses, plasma exposure of MK-8527 increased in an approximately dose-proportional manner, and intracellular exposure of MK-8527-TP (PBMCs) was slightly less than dose proportional over 5–200 mg. Administration of MK-8527 with a meal resulted in lower systemic exposure (AUC0-168) and slower absorption (Cmax) than MK-8527 without a meal. After administration of MK-8527 with a meal, the true geometric mean Cmax and AUC0-168 ratios [Day 15/Day 1] was 0.56 and 0.54, respectively. In trial A, maximum observed plasma concentration (Cmax) was 110–1120 hours, and apparent half-life (t1/2) was 10–24 hours, and apparent half-life (t1/2) was similar in CAB200 IM. CAB t1/2 in C1 was longer than in C2; both were lower than CAB200 IM. CAB t1/2 in C1 was longer than in C2; both were longer than CAB200 IM, even though some pts have not reached terminal phase due to long t1/2. PK simulations predict a CAB400 SC/IM dose interval of ≥4 months achieves similar exposure to the approved CAB200 IM. Injection site reactions (ISRs) occurred in all pts dosed SC in part A (22/22) with a dose-related trend for increased ISR grades. A sentinel pt in cohort A experienced a drug-related serious adverse event of injection site erythema with necrosis. ISRs in C1 (8/8 pts) and C2 (3/8 pts) were grade 1 or 2.

Conclusion: Safety and PK results from part A indicate low potential to achieve less frequent dosing with CAB200 and HuPiH20. The new CAB400 formulation (SC and IM) exhibits favorable safety and PK permesability with dose intervals of ≥4 months and is in ongoing clinical development.

Table: Pharmacokinetics and Safety: CAB400 (SC and IM) and CAB200 (A1 and A2)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAB400 (SC and IM)</th>
<th>CAB200 (A1 and A2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µM)</td>
<td>110–1120 hours</td>
<td>110–1120 hours</td>
</tr>
<tr>
<td>AUC0-168 (µM·h)</td>
<td>271–272 hours</td>
<td>271–272 hours</td>
</tr>
<tr>
<td>Grade 1/2 events (%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Grade 3 or 4 events (%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*Calculated for pts who have reached terminal phase at least 15 days. Evaluated for all, meaning 27 have not reached terminal phase dose for long t1/2. A drug-related serious adverse event of injection site erythema with necrosis.

130 Phase I Study of Cabotegravir Long-Acting Injectable Formulations Supports ≥4-Monthly Dose Interval

Kelong Han1, Ronald D’Amico, Jeng Sievers1, Darin Brimhall1, Brian Spears1, Dale Taylor1, David Dorey1, Paul Bent2, Lisa Morgan3, Russ P. Carstens1, Xiaowei Zang2

GSK, Collegeville, PA, USA, 1ViiV Healthcare, Durham, NC, USA, 1ViiV Healthcare, Brentford, United Kingdom, 1Thermo Fisher Scientific, Las Vegas, NV, USA, 1Thermo Fisher Scientific, Austin, TX, USA, 1Thermo Fisher Scientific, Dallas, TX, USA, 1GSK, Mississauga, ON, Canada, 1GSK, Brentford, United Kingdom, 1ViiV Healthcare, Brentford, United Kingdom

Background: Long-acting cabotegravir (CAB) administered intramuscularly (IM) every 2 months (Q2M) is approved for HIV-1 prevention; CAB and rilpivirine administered IM monthly or Q2M is approved for HIV-1 treatment. To support less frequent dosing, we evaluated safety and pharmacokinetics (PK) of the approved CAB 200 mg/mL (CAB200) formulation administered subcutaneously (SC) with recombinant human hyaluronidase PH20 (HuPiH20) and a new CAB 400 mg/mL (CAB400) formulation administered SC or IM without HuPiH20.

Methods: This is an ongoing, open-label, single-dose, dose-escalation, phase 1 study (NCT05148868) in healthy adults with 2 sentinel participants (pts) per cohort. In part A, HuPiH20 (10,000 IU) and CAB200 (A1, 800 mg; A2, 1600 mg; A3, 3200 mg; 4 to 16 mL) were sequentially co-administered SC (abdominal); CAB200 (A3, 3200 mg; 4 to 16 mL) was also administered SC (abdominal); CAB200 (A1, 800 mg; A2, 1600 mg; A3, 3200 mg; 4 to 16 mL) was also administered IM (glutes medium; C2). To evaluate potential CAB400 dosing regimens, CAB PK profiles were simulated using an established CAB200 IM population PK model modified based on observed PK data in part A.

Results: To date, 38 pts total received CAB (Table); 61% were male sex at birth, and 61% were non-white. Median age, weight, and BMI were 37.5 years, 74.7 kg, and 26.7 kg/m², respectively. In part A, maximum observed plasma concentration (Cmax) and area under the plasma concentration–time curve from 0 to infinity (AUC0-∞) increased with dose proportionally and were higher than CAB200 IM, indicating potentially increased bioavailability, while t1/2 was similar to CAB200 IM. Cmax in C1 was lower than in C2; both were lower than CAB200 IM. CAB t1/2 in C1 was longer than in C2; both were longer than CAB200 IM, even though some pts have not reached terminal phase due to long t1/2. PK simulations predict a CAB400 SC/IM dose interval of ≥4 months achieves similar exposure to the approved CAB200 IM. Injection site reactions (ISRs) occurred in all pts dosed SC in part A (22/22) with a dose-related trend for increased ISR grades. A sentinel pt in cohort A experienced a drug-related serious adverse event of injection site erythema with necrosis. ISRs in C1 (8/8 pts) and C2 (3/8 pts) were grade 1 or 2.

Conclusion: Safety and PK results from part A indicate low potential to achieve less frequent dosing with CAB200 and HuPiH20. The new CAB400 formulation (SC and IM) exhibits favorable safety and PK permesability with dose intervals of ≥4 months and is in ongoing clinical development.

Table: PPV for initial reactive visits with adjudicated HIV status (177 total, 129 confirmed HIV-positive) by treatment arm from HPTN 083

<table>
<thead>
<tr>
<th>HIV-positive total (N=129)</th>
<th>HIV-negative total (N=48)</th>
<th>Difference in PPV (%) (CAB200 vs CAB400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAB200</td>
<td>CAB400</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>100%</td>
<td>54%</td>
</tr>
<tr>
<td>0%</td>
<td>0%</td>
<td>-54%</td>
</tr>
</tbody>
</table>

*Calculated for pts who have reached terminal phase at least 15 days. Evaluated for all, meaning 27 have not reached terminal phase dose for long t1/2. A drug-related serious adverse event of injection site erythema with necrosis.
132 COVID Incidence and Severity in Persons With Reinfection vs Post-Vaccination Breakthrough Infection

Adeel A. Butt, Peng Yan, Obaid S. Shaikh

1Vanderbilt University, Nashville, TN, USA
2Pittsburgh Healthcare System, Pittsburgh, PA, USA
3South Africa, Durban, South Africa
4Malawi College of Medicine-Johns Hopkins University Research Project, Blantyre, Malawi
5South African Medical Research Council, Cape Town, South Africa
6Fred Hutchinson Cancer Center, Seattle, WA, USA
7National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA
8South African Medical Research Council, Cape Town, South Africa
9Centre for the AIDS Programme of Research in Sub-Saharan Africa, Durban, South Africa

**Background:** COVID-19 vaccine effectiveness wanes over time. Natural infection also provides a high level of protection from reinfection and from severe, critical, or fatal disease. While both natural and vaccine-induced immunity are highly protective, reinfections after natural infection and breakthrough infections after vaccination have been reported. Our aim was to determine the incidence rate and rate of severe/critical COVID-19 among those with breakthrough infection after full vaccination compared with reinfection among unvaccinated persons.

**Methods:** The study was conducted using the US Department of Veterans Affairs (VA) COVID-19 databases. Individuals with a first confirmed infection >14 days after 2 doses of Pfizer or Moderna vaccine were matched 1:1 to individuals with a second confirmed infection in unvaccinated individuals >14 days after the first infection. Severe/critical disease, defined as admission to an intensive care unit, mechanical ventilation, or death within 28 days of the index test positive date, was compared among the two groups.

**Results:** We identified 55,251 matched pairs. Median age was 56 years, 88% were male, and 75% were White, median Charlson Comorbidity Index was 1, 31% were symptomatic at baseline. The incidence rate of breakthrough infection among vaccinated individuals was 0.29/1,000 person-years (PY; 95% CI 0.28-0.3) of follow up. Rate of reinfection among unvaccinated was 0.38/1,000 PY (95% CI 0.37-0.39). The probability of remaining free of severe/critical disease was higher among vaccinated individuals with breakthrough infection compared with individuals with reinfection. (Figure)

**Conclusion:** Incidence and severity of breakthrough infection and the risk of severe/critical disease after such infection is lower among vaccinated individuals compared with incidence and severity of reinfection among unvaccinated individuals.

<table>
<thead>
<tr>
<th>Reinfection vs Post-Vaccination Breakthrough Infection</th>
<th>Incidence Rate</th>
<th>Sensitivity Analysis #1</th>
<th>Sensitivity Analysis #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Case Model</td>
<td>0.29 (0.28-0.3)</td>
<td>0.38 (0.37-0.39)</td>
<td>0.37 (0.36-0.38)</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.28-0.3</td>
<td>0.37-0.39</td>
<td>0.36-0.38</td>
</tr>
<tr>
<td><strong>Note:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity analysis #1: matched participants as having an incident STI (ever) or never; Sensitivity analysis #2 contrasted STI status back to the last STI negative test (assuming rapid STI treatment to interrupt the transmission cycle). CI=confidence interval.

**Background:** CoVPI 3008 (Ubuntu), the largest multicenter phase 3/4 trial of mRNA vaccines in sub-Saharan Africa, was designed to assess the safety of mRNA-1273, the effectiveness of hybrid versus vaccine immunity, and SARS-CoV-2 viral persistence among people with HIV (PWH).

**Methods:** We enrolled adults aged ≥18 years living with HIV or another comorbidity associated with severe Covid-19. Previously vaccinated individuals were excluded. Participants were assigned vaccinations at enrollment only or enrollment and month 1 based on whether their baseline point-of-care SARS-CoV-2 serostatus was positive (hybrid immunity) or negative (vaccine immunity). For the first 6 months of follow-up, the association between hybrid versus vaccine immunity with Covid-19 and severe Covid-19 was assessed using calendar-time-scale Cox regression models and counterfactual cumulative incidence methods.

**Results:** Between December 2021 and September 2022, 14237 participants were enrolled, of which 11681 PWH (median age 39 years, 77% female) were included in the Full Analysis Subset (FAS). Among PWH, the median CD4 count was 635 cells/mm³ (IQR 423-866), 769 (6.6%) had a CD4 count <200 cells/mm³, 2157 (18.5%) had a detectable viral load (≥50 copies/ml), and 14.5% were not on ART. Retention was high (>95%) through the month 6 visit. The vaccinations were well tolerated. Among PWH, the 6-month cumulative incidence (Fig1A) in the vaccine immunity and hybrid immunity groups, respectively, was 7.77% (95% confidence interval [CI] 6.21 to 9.23) and 3.90% (95% CI 3.30 to 4.49) for SARS-CoV-2 infection, 3.40% (95% CI 2.30 to 4.49) and 2.02% (95% CI 1.61 to 2.44) for Covid-19, and 0.32% (95% CI 0.59 to 0.63) and 0.048% (95% CI 0 to 0.1) for severe Covid-19. The covariate-adjusted hazard rate was 42% lower in the hybrid immunity group for Covid-19 (hazard ratio [HR] 0.58; 95% CI 0.44 to 0.77; p<.001) and 73% lower (HR 0.27; 95% CI 0.07 to 1.04; p=0.056) in the hybrid immunity group for severe Covid-19 (Fig1B). Twenty-two individuals had persistent SARS-CoV-2 infection ≥50 days, which was more often among those with prior TB infection, HIV viiremia, or low CD4 count.

**Conclusion:** Individuals with hybrid immunity, even if living with HIV, were more effectively protected from Covid-19 and severe Covid-19 compared to those with vaccine immunity. Our results also highlight the importance of better understanding the role of persistent infections in transmission and in the emergence of new variants of concern through mutation evolution.

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SARS-CoV-2 Viral Clearance and Evolution Varies by Extent of Immunodeficiency

Yijia Li1, Manish C. Choudhary2, James Regan1, Julie Boucau1, Anusha Nathan1, Tessa Speidel3, Mary Y. Liew4, Gregory E. Edelstein1, Michael S. Seaman1, Gaurav D. Gaiha5, Mark J. Siedner1, Amy K. Barczak2, Jacob E. Lemieux1, Jonathan Z. Li1, for the POSITIVES Study Team

1University of Pittsburgh, Pittsburgh, PA, USA; 2Brigham and Women’s Hospital, Boston, MA, USA; 3Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA; 4Beth Israel Deaconess Medical Center, Boston, MA, USA; 5Massachusetts General Hospital, Boston, MA, USA

Background: Despite vaccination and antiviral therapies, immunocompromised individuals are at risk for prolonged SARS-CoV-2 infection, but the immune defects that predispose to persistent COVID-19 remain incompletely understood.

Methods: Participants enrolled in the POST-VaccInaTIon Viral CharactEristics Study (POSITIVES), a prospective cohort study enrolling participants with confirmed SARS-CoV-2 infection. Participants were categorized based on the extent of immunocompromise into severe hematologic malignancy/ transplant group (S-HT), severe autoimmune/B-cell deficient (S-A), non-severe immunodeficiency (NS), and non-immunocompromised (None). Longitudinal nasal SARS-CoV-2 levels were measured with a quantitative PCR assay and viable virus levels were evaluated by viral culture. Neutralizing antibody, binding antibody to nucleocapsid, and T cell profiling (enzyme-linked immunosorbent spot [ELISpot] and Spike-specific proliferation assay) were performed in a subset of participants with available blood samples.

Results: The median time to nasal viral RNA and culture clearance in the severe hematologic malignancy/transplant group (S-HT) were 72 and 40 days, respectively, which were significantly longer than clearance rates in the severe autoimmune/B-cell deficient (S-A), non-severe, and non-immunocompromised groups (P=0.002 Figure 1A and P=0.001 Figure 1B). Individuals with B-cell deficiency (S-A group) had an intermediate risk of persistent infection. Participants who were severely immunocompromised (S-HT and S-A) had greater SARS-CoV-2 evolution and a higher risk of developing antiviral treatment resistance. Both S-HT and S-A participants had severely diminished SARS-CoV-2-specific humoral responses. In contrast, S-A group had the highest level of SARS-CoV-2-specific CD4+ and CD8+ T cell proliferation response among all groups, while S-HT demonstrated neither antibody maturation nor increased T cell proliferation response to Spike peptide pools. NS and non-immunocompromised participants showed both increasing neutralizing antibody levels (until a plateau ~25-30 days post symptom onset) and SARS-CoV-2-specific T cell responses.

Conclusion: Our study demonstrated a hierarchy of immunocompromised conditions that increase the risk of delayed viral clearance and SARS-CoV-2 evolution, with the highest risk in those with severe hematologic malignancy/ transplant. The findings may be explained by the suppression of both SARS-CoV-2-specific B and T cell responses.

Analysis of Emergent SARS-CoV-2 Antiviral Resistance and Its Association With Virologic Rebound

Trevor J. Tamura1, Fizah Yousuf1, Manish C. Choudhary2, Rinki Deo1, Anabela Navarrete Gomez1, Gregory E. Edelstein2, Julie Boucau1, Dessie Tien1, Tammy D. Vyas1, Robert W. Shafer2, Mark J. Siedner1, Amy K. Barczak2, Jacob E. Lemieux1, Jonathan Z. Li1, for the POSITIVES Study Team

1Brigham and Women’s Hospital, Boston, MA, USA; 2Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA; 3Massachusetts General Hospital, Boston, MA, USA; 4Stanford University, Stanford, CA, USA

Background: Nirmatrelvir-ritonavir (N-R) and remdesivir (RDV) are SARS-CoV-2 antivirals that reduce the risk of hospitalization and progression to severe COVID-19. N-R and RDV resistance have been described previously, but the frequency and risk factors for emergent drug resistance remains unclear.

Methods: We enrolled non-hospitalized participants with acute SARS-CoV-2 infection into the POSITIVES study, a prospective observational cohort, where we collected anterior nasal swabs thrice weekly during the first two weeks after diagnosis. From these samples, we performed deep sequencing of nsp5 among N-R treated (n=53) and untreated (n=42) participants and nsp12 among RDV treated (n=14) participants. We compared the incidence of emergent N-R resistance between N-R treated and untreated participants and evaluated its association with post-treatment virologic rebound, while also characterizing emergent RDV resistance.

Results: Compared with untreated individuals, those treated with antivirals were older, more immunosuppressed, and had received more COVID-19 vaccinations, reflecting guidelines for N-R and RDV use. Emergent N-R mutations targeted to at least confer moderate resistance (≥2.5-fold reduced susceptibility to N-R in vitro) were detected more often in those who received N-R than those who did not (5/53 [9%] vs 0/42 [0%], p=0.06). However, these mutations (E166V, H172Y, Q189K, P252L) were detected at low frequencies, with all but one mutation present in <25% of the viral population. Additionally, for those with detectable viral loads at follow-up timepoints after cessation of treatment, all (4/4) of the emergent resistance mutations subsequently reverted to wild type. Virologic rebound occurred in 28% (15/53) of participants receiving N-R, but there was no difference in resistance emergence in those who experienced virologic rebound compared to those who did not (2/15 [13%] vs 3/38 [8%], p=0.6). Emergent RDV resistance mutations were detected in two immunosuppressed participants (14%). However, similar to the N-R mutations, all four mutations (V166L, N198S, V792I, M794I) were low-frequency and reverted to wild type at successive timepoints.

Conclusion: Mutations that confer resistance emerge with N-R and RDV treatment, but they are transient, present at minor frequencies, and are not associated with virologic rebound. These data suggest that the risk of widespread dissemination of significant drug resistance to N-R and RDV remains low.
Molnupiravir Does Not Increase 3CLpro Resistance Mutations When Co-Administered With Nirmatrelvir/r

Shuntai Zhou,1 Nathan Long,1 Kyle Rosenek1, Michael A. Jarvis,1 Heinz Feldmann,1 Ronald Swanstrom2
1University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 2National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA

Background: Current treatments for SARS-CoV-2 rely on direct-acting antivirals including the 3CLpro inhibitor nirmatrelvir/r (NMV/r) and the mutagenic nucleoside analog molnupiravir (MOV). There have been concerns regarding the use of MOV including the generation of new viral variants. In a previous study, we compared the antiviral effect of MOV or NMV/r alone, or the co-administration of both in a SARS-CoV-2 macaque model. We showed an additive effect of the two antivirals on several markers of disease. In this follow-up study, we investigated the mutation profiles of SARS-CoV-2 in samples collected in the previous study.

Methods: Twenty macaques received the oral treatment of MOV, NMV/r, combination therapy or vehicle control (5 in each group) starting 12 hours after exposure to SARS-CoV-2, and they were followed for 4 days before necropsy. Viral RNA was extracted from lung tissue samples at necropsy. Primer ID sequencing approach was used to sequence nsp5 (3CLpro), a portion nsp12 (RdRp) and the S gene receptor binding domain (S-RBD). Substitutions with a FDR-adjusted p value of less than 0.05 were considered as true mutations. This work was partially funded by the Intramural Research Program, NIAID.

Results: In the MOV group, the overall substitution rate was approx. 0.05%, significantly higher than other groups. The mutation profile was driven by the increase of C-to-U and G-to-A mutations. The overall substitution rates were significantly increased in the MOV+NMV/r group compared with NMV/r or vehicle groups, but lower than MOV group (Fig 1A). We further studied the impact of MOV on the potential development of resistance mutations on 3CLpro against NMV. We did not observe any mutation hotspots in this region beyond those mediated by MOV. Mutations identified in the MOV group were largely distinct in frequency from those in the combination group. We assessed the amino acid codon changes at four positions (L50F, E166A, E166V, and L167F) that have been reported to contribute to NMV resistance. We did observe an increase in the abundance of L50F in the MOV group but it was not further enriched in the combination group (Fig 1B).

Conclusion: Our results suggest that co-administration of NMV/r lowered the magnitude of the mutagenic effect of MOV against SARS-CoV-2, likely due to the additive effect of the combination therapy on reduced rounds of viral replication. There is no evidence that combination therapy potentiated selection for NMV resistance mutations during 4 days of treatment. The figure, table, or graphic for this abstract has been removed.

Mini-Lecture: Progress in Understanding the Mechanisms of Long COVID

Annukka A. Antar
The Johns Hopkins University, Baltimore, MD, USA

Background: Millions of people across the globe have experienced new or persistent symptoms for 3 or more months following COVID-19, a syndrome termed long COVID. The US Census estimates that over 15% of US adults have ever had long COVID. Investigations are underway to understand the biologic mechanisms of long COVID and develop effective therapeutics. This mini-lecture will highlight the progress made in the past year on understanding the pathogenesis of long COVID.

Multimodal Assessment of Antigen Persistence in the Post-Acute Phase of SARS-CoV-2 Infection

Michael I. Peluso1, Sarah Goldberg, Zoe Swank, Brian H. Lafranchi, Scott Liu, Thomas Dalhuisen, Badri Virovanathan, Ma Somsouk, J. D. Kelly, Steven G. Deeks, Zoltan Laszik, David Walt1, Jeffrey Martin, Timothy J. Henriot, for the LIINC Study Team
1University of California San Francisco, San Francisco, CA, USA, 2Brigham and Women’s Hospital, Boston, MA, USA

Background: Although RNA viruses like SARS-CoV-2 are considered transient, viral components can persist beyond the acute phase due to various virologic and immunologic factors. Recent studies have suggested that SARS-CoV-2 antigens may persist following COVID-19 but were limited by a lack of comparison to true negative control samples.

Methods: We assessed viral persistence in two ways: (1) Single molecule array (Simoa) assays for SARS-CoV-2 spike, S1, and nucleocapsid antigen in plasma from 171 individuals in the post-acute phase of SARS-CoV-2 infection and 250 pre-pandemic control samples, and (2) RNAsecope assessing SARS-CoV-2 spike RNA in situ in rectal tissue obtained via flexible sigmoidoscopy in 5 individuals between 90 and 676 days post-COVID (without reinfection), with H&E and immunohistochemical visualization of CD3 and CD68 to localize viral RNA signals within tissue regions and immune cell types.

Results: In plasma, compared to the proportion of antigen positivity in pre-pandemic controls (2.9%), detection of any SARS-CoV-2 antigen was more frequent across all post-acute COVID-19 time periods (3-6 months: 12.6%, p<0.001; 6-10 months, 10.7%, p=0.0002; 10-14 months, 7.5%, p=0.017; a). These differences were driven by spike for up to 14 months and nucleocapsid in the first 6 months after infection. Hospitalization for acute COVID-19 was associated with detectable antigen in the post-acute phase (OR 2.27, p=0.054) and strongly associated with detectable N antigen (OR 11.82, p=0.001). In gut, RNAsecope revealed readily detectable SARS-CoV-2 RNA in multiple cells from all rectal tissue regions surveyed from 4/5 individuals (b), except for one who had rare RNA+ cells detected in 1/3 regions. Nearly all RNA+ cells were detected in the lamina propria, without an epithelial signal. A small percentage of RNA+ cells expressed CD68, a monocyte marker, but many RNA+ cells did not express CD68 and none expressed CD11. In 3/4 samples with readily detected RNA, the signal was associated with macrophage-dense areas.

Conclusion: Our findings provide strong evidence that SARS-CoV-2 antigens can persist beyond the period of acute illness. The observation that 7-13% of plasma samples for over a year following initial SARS-CoV-2 infection contain detectable viral antigens, which are potentially immunogenic, has significant implications given the sheer number of people infected with SARS-CoV-2 to date. Work to determine if persistent antigen contributes to post-acute sequelae such as Long COVID is needed.

HIV-1 Transcription Start Sites Usage and Its Impact on Unspliced RNA Functions In Vivo

Saifual Islam1, Zetao Cheng1, Olga Nikolaitchik2, Robert Gorelick1, Vinay K. Pathak1, Frank Maldarelli1, Wei-Shau Hu1
1National Cancer Institute, Frederick, MD, USA, 2Frederick National Laboratory for Cancer Research, Frederick, MD, USA

Background: HIV-1 unspliced RNA plays two crucial roles in viral replication: it is packaged into particles to serve as viral genome, and it is translated to generate Gag/Gag-Pol polyproteins required for virus assembly and replication. Studies using cell culture systems demonstrated that HIV-1 transcription can initiate at three conserved consecutive guanosines located at the junction of U3 and R regions, producing RNAs containing three, two, or one guanosine at the 5’ end, referred to as 3G, 2G, and 1G, respectively. Furthermore, 1G RNA selectively packaged over 3G RNA into viral particles to serve as virion genome, suggesting these almost identical HIV-1 RNA species differ functionally. To investigate whether HIV-1 uses multiple transcription start sites and preferentially packages a specific RNA species in vivo, we examined HIV-1 unspliced RNA in paired PBMC and plasma samples collected from infected individuals.

a) Prevalence of SARS-CoV-2 antigen (Spike, N, or S1) detection in true negative versus post-COVID samples.

b) Representative slide showing SARS-CoV-2 Spike RNA detected in gut lamina propria in association with CD68+ cells 2 years post-COVID using RNAsecope and immunohistochemistry.
Methods: To study HIV-1 transcription start site usage, we established a next-generation sequencing (NGS)-based 5' rapid amplification of cDNA end (5' RACE) method. The accuracy and reproducibility of this assay were verified using in vitro-transcribed RNAs as templates and by determining the 5' context of HIV-1 unspliced RNA in multiple biological replicates of infected cells and virions. Using samples with characterized HIV-1 transcripts, we further determined the copy number of HIV-1 unspliced RNA required to obtain accurate measurement.

Results: To study HIV-1 transcription start site usage in vivo, we used NGS-based 5' RACE to analyze RNA samples isolated from PBMCs of infected individuals. In most samples, there are several HIV-1 unspliced mRNA species with varied 5' ends, indicating that multiple transcription start sites are used. Furthermore, 3G RNA is often the most abundant RNA species in PBMC samples. We have also examined the HIV-1 RNA species in corresponding plasma samples. Intriguingly, the distribution of HIV-1 unspliced RNA species in the plasma is distinct from that of the PBMCs: 1G RNA is the most abundant RNA species in the patient plasma samples, consistent with preferential packaging of 1G RNAs into virions.

Conclusion: Our preliminary results indicate that in vivo, HIV-1 uses heterogeneous transcription start sites to generate multiple unspliced RNA species. Furthermore, these 99.9% identical HIV-1 unspliced RNA species differ functionally. The 1G RNA is preferentially selected as virion genome to transfer genetic information to the progeny.

Investigation of the Functional Role of DDX42 in HIV-1 Viral RNA Splicing
Xiao Lei1, Ann Emery1, Peng Zhang1, Arjun Kanjapane2, Ronald Swanstrom3, Paul D. Bieniasz1

1The Rockefeller University, New York, NY, USA; 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 3University of Maryland, Baltimore, MD, USA

Background: Understanding the mechanism of HIV-1 viral RNA splicing could lead to the discovery of therapeutic potential drugs that target HIV-1 viral splicing pathways. HIV-1 splicing is regulated by cis-acting regulatory sequences and trans-acting splicing factors. We aim to identify host splicing factors that play roles in HIV-1 splicing through high-throughput assays.

Methods: We generated a stable HEK-293T cell line harboring an HIV-1 splicing reporter which contains GFP in Gag position and RFP in Nef position in the viral genome. This reporter indicates the expression of unspliced viral RNAs and completely spliced viral RNAs through the expression of GFP and RFP respectively. We introduced genome-wide CRISPR knockout sgRNA library into the splicing reporter cell line and sorted cells with significantly higher or lower GFP/RFP expression through fluorescence-activated cell sorting. We analyzed enriched sgRNA guides in each sorted cell population to identify genes whose disruption might affect HIV-1 viral RNA splicing.

Results: We identified an RNA helicase DDX42, whose disruption caused an increase in completely spliced viral RNA species and a decrease in incompletely spliced viral RNA species. HIV-1 viral RNA splicing assays based on next generation sequencing show that the usage of specific HIV-1 viral RNA splicing acceptors is affected in DDX42 knockout cells. For example, the usage of viral splicing acceptor A3 is increased significantly upon DDX42 knockout, which is also reflected in the increase of Tat protein expression in DDX42 knockout cell clones in western blot analysis. RNAseq analysis also reveals a role for DDX42 in the regulation of alternative splicing for a subset of host genes.

Conclusion: DDX42 plays a role in the regulation of HIV-1 viral RNA splicing through up- and down- regulation of the usage of specific viral RNA splicing acceptors.

Structural Basis of Translation Inhibition by MERS-CoV Nsp1 Reveals a Conserved Mechanism for β-CoVs
Michael Vetick, Swapnil Devarkar, Shrvani Balaji, Ivan Lomakin, Yong Xiong

Yale University, New Haven, CT, USA

Background: Since 2002, three deadly human betacoronaviruses (β-CoVs) have emerged: SARS-CoV, MERS, and SARS-CoV-2, however our therapeutic arsenal remains inadequate to restrict current and novel β-CoVs. All β-CoVs encode non-structural protein 1 (Nsp1), an essential pathogenicity factor that potently inhibits host gene expression. Three distinct mechanisms are proposed for Nsp1: two cytosolic involving the ribosome and one nuclear stopping mRNA export. Also, across β-CoVs Nsp1 has very low amino acid conservation. Previous literature suggests MERS Nsp1 function is restricted to the nucleus and does not bind 40S ribosome, contrasting SARS-CoV-2 Nsp1. However, due to conserved structure prediction, we hypothesized that Nsp1 function is highly conserved across β-CoVs.

Methods: Nsp1's effects on translation were evaluated in vitro. HeLa cytoplasmic extracts were incubated with an exogenous luciferase reporter mRNA and recombinant Nsp1. Nsp1-40S ribosome binding was evaluated via fluorescence polarization. Fluorescein was conjugated to Nsp1 and incubated against a range of 40S ribosomes. A high-resolution structure of MERS Nsp1 binding the 40S ribosome was obtained by single particle cryo-EM of in vitro assembled complexes.

Results: Contrary to previous reports, MERS Nsp1 robustly inhibits the translation of luciferase mRNA in vitro. Compared to MERS Nsp1, SARS-CoV-2 Nsp1 inhibits translation at the ribosomal level more efficiently. However, fluorescence polarization showed that MERS and SARS-CoV-2 Nsp1 bind the 40S ribosome with very similar Kd's, 40mM and 27mM respectively. We resolved a 2.6 Å structure of MERS Nsp1 binding the 40S ribosome. The CTD of MERS Nsp1 adopts a helix-turn-helix motif binding in the mRNA entry channel of the 40S subunit interacting with the 18S rRNA and ribosomal proteins u53 and u55. Mutating this binding interface completely ablates Nsp1 function in vitro.

Conclusion: We show divergent Nsp1 proteins exhibit a remarkably conserved mechanism by targeting the 40S ribosome to restrict host gene expression. Since SARS-CoV-2 and MERS Nsp1 have similar Kd's for the 40S ribosome, we speculate the increased potency of SARS-CoV-2 Nsp1 is due to effects beyond initial binding to the 40S ribosomal subunit. Diversified therapeutics targeting multiple stages of the viral life cycle will be critical for containing β-CoV outbreaks. We present that Nsp1 of β-CoVs is an essential pathogenicity factor and an attractive target for therapeutic intervention which can broadly restrict β-CoVs.
EHMT2, CEACAM3, CC2D1B, RHOA and HMOX1 provide significant advantages for viral replication fitness. Knock-out studies confirmed that GRN, CEACAM3 and CIT1 inhibit HIV-1 in primary CD4+ T cells. Several factors were identified with high reproducibility in our virus-driven screen but not in common overexpression or knock-down assays. Finally, lack of the nef gene increased selection of sgRNAs targeting SERINC5 and identified IFIT6 as putative Nef target. Subsequent analysis confirmed that Nef attenuates the inhibitory effects of IFIT6.

Conclusion: We established an innovative, robust and highly versatile virus-driven approach that allows the identification of antiviral factors by turning HIV-1 into “traitors” revealing their cellular opponents.

143 Immunoregulatory Pathways Predict Mortality More Strongly in People With Versus Without HIV
Samuel R. Schnittman1, Rebecca Abelmann1, Gabrielle B. Beck-Engeser2, Noah Aquino2, Gabrielle C. Ambayec1, Carl Grunfeld1, Edward Cachay1, Joseph J. Eron3, Michael S. Saag4, Robin M. Nance5, Joseph A. Delaney5, Heidi M. Crane6, Adam Olsner1, Peter W. Hunt1, for the CFAR Network of Integrated Clinical Systems (CNICS) Network
1Massachusetts General Hospital, Boston, MA, USA, 2University of California San Francisco, San Francisco, CA, USA, 3University of California San Diego, San Diego, CA, USA, 4University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 5University of Alabama at Birmingham, Birmingham, AL, USA, 6University of Washington, Seattle, WA, USA

Background: While people with HIV (PWH) on suppressive antiretroviral therapy (ART) have higher levels of inflammation and are at greater risk for morbidity and mortality than the general population, the immunologic pathways most strongly linked to this excess HIV-associated risk remain incompletely characterized.

Methods: The first available plasma sample after 1/1/2010 was selected from a random sample of all PWH from 8 CNICS sites with ≥6 months of ART-mediated viral suppression. Plasma was assessed for 363 detectable inflammatory proteins (Olink Inflammation I Explore Panel). The relationship between each protein and all-cause mortality was assessed via Cox proportional hazards modeling, adjusted for VACS index (1.0) and CNICS site, controlling for the false discovery rate (FDR) via the Benjamini-Hochberg method. To identify pathways more relevant in PWH than in the general population, adjusted hazard ratios (aHR) were compared to published UK Biobank (UKB) data, which reported the association between the same Olink panel and all-cause mortality in 47,600 participants from the general population via FDR-corrected multivariate Cox modeling.

Results: Among 922 PWH in CNICS, median age was 47 and 18% were women. Median current and nadir CD4 counts were 579 cells/mm$^3$ and 245 cells/mm$^3$, respectively. Over a median follow-up time of 9 years, 103 deaths occurred. After adjustment and FDR correction, 147 (40%) proteins were associated with an increased (N=140) or decreased (N=7) hazard of death in CNICS (all FDR-corrected P<0.05, Figure A). Compared to the same proteins assessed in UKB, higher levels of 48 proteins were uniquely associated with increased mortality in CNICS (N=21) or had an aHR at least 50% higher among PWH than in the UKB general population (N=27) (Figure B). While some of these HIV-associated mortality predictors were pro-inflammatory (IFNγ, IFNγR1, IL-18R1, and IL-15), the largest cluster comprised immunoregulatory proteins linked to suppressing T, NK, and myeloid cell activation (CD276, CD58, CD48, ITGB6, CLEC4G, and KLRD1) or pro-fibrotic and endothelial cell regulatory processes.

Conclusions: The pathways most strongly linked to this excess HIV-associated risk remain incompletely characterized.
levels of IL-6 (p=0.0015). In participants with detectable atherosclerotic plaque, levels of sgp120, anti-cluster A Abs, and their combination were associated with increased volume of atherosclerotic plaques (p=0.01, 0.018, and 0.006, respectively).

Conclusion: sgp120 could act as a pan toxin, causing immune dysfunction and sustained inflammation in a subset of PLWH, contributing to the development of premature comorbidities.

145 Rapid Emergence and Adaptive Evolution of SARS-CoV-2 Variants in Advanced HIV Infection

Sung Ree Ko1, Pierre Radecki1, Frida Belinsky1, Jinal Bhiman1, Susan Meiring1, Jackie Kleyman2, Daniel Amaoko1, Margaret Lucas1, Vanessa Guerra1, Tatiana Bylund1, Nicole Wolter2, Stefano Tempia3, Anne von Gottberg4, Cheryl Cohen5, Eli Bonitz6

1National Institutes of Health, Bethesda, MD, USA, 2National Institute for Communicable Diseases, Johannesburg, South Africa

Background: Prolonged SARS-CoV-2 RNA shedding and intra-host evolution in people with HIV (PWH) suggested that SARS-CoV-2 variants, including variants of concern (VOCs), may preferentially arise in PWH. Nonetheless, the evolutionary processes remain incompletely understood due to consensus-based genetic characterization of intra-host virus from short-read whole genome sequencing. Alternatively, high-throughput single-genome amplification and sequencing (HT-SGS), which enables detection of unique linked groupings of mutations (i.e. haplotypes) at the level of single genomes, is more suitable for estimates of intra-host population diversity and allows a better understanding of evolutionary relationship among viruses.

Methods: We sequenced SARS-CoV-2 spike genes in nasal swabs of longitudinal sample sets from 25 people without HIV (PWH) and 22 PWH, who were subgrouped on the basis of CD4 T cell counts (CD4 counts). We developed HT-SGS, which used Pacific Biosciences single molecule, real-time technology (SMRT) coupled with unique molecular identifiers (UMIs) of virus genome sequences, to generate up to ~1000 single-copy sequences per sample with high accuracy.

Results: Intra-host spike gene diversity was significantly higher in PWH with CD4 counts <200 cells/μL than in the other subgroups. These individuals had a median of 3.5 secondary Pango lineages/person, while PWH had showed no secondary lineages. Through longitudinal analysis, remarkable features of SARS-CoV-2 dynamics were observed in PWH with CD4 counts <200 cells/μL, including 1) high early diversity, beginning shortly after COVID-19 symptom onset, 2) rapid changes in frequency of the most abundant haplotypes, and 3) large changes in population haplotype composition over time. Intra-host polymorphisms in PWH with CD4 counts <200 cells/μL included greater numbers of synonymous (reflecting more virus replicative cycles) and nonsynonymous mutations (often overlapping with defining mutations of VOCs) than other subgroups, indicating a high mutational burden. In addition, we found that patterns of gene evolution in PWH with CD4 counts <200 cells/μL resulted from adaptation of the virus to the host by selective forces (positive selection).

Conclusion: These reveal unique virus genetic aspects of SARS-CoV-2 infections in people with advanced HIV infection that markedly increase the risk for generation of new variants. Our results suggest that HIV treatment with antiretroviral therapy can help limit intra-host SARS-CoV-2 persistence and evolution.

146 Temperature-Dependent SARS-CoV-2 Spike-ACE2 Interaction Is Associated With Viral Transmission

Mehdi Benlarbi1, Shihui Ding1, Etienne Bélanger1, Alexandre Touzini1, Halima Medjahed1, Raphael Poujal1, Omar El-Ferri1, Yuxia Bo1, Julie Hussin1, Judith Furdac1, Marzena Pazgier1, Ines Levade1, Cameron Abrams2, Marceline Côté1, Andréas Frizz1

1Université de Montréal, Montreal, Canada, 2Institut de Cardiologie de Montréal, Montreal, Canada

Background: The persistent evolution of SARS-CoV-2 gave rise to a wide array of variants harboring new mutations in their Spike glycoprotein. We previously demonstrated that temperature modulates the interaction between SARS-CoV-2 Spike and its host receptor ACE2, with low temperature increasing ACE2 binding affinity and viral entry. Here we characterized the latest Omicron subvariants and evaluated whether this property is associated with viral transmission.

Methods: We first tested the capacity of plasma from 18 individuals who received a fifth dose of bivalent (BA.1 or BA.4/5) mRNA vaccine to recognize and neutralize Spikes from 13 recent Omicron subvariants. We also tested the susceptibility of Spikes to cold inactivation and measured their processing. We also measured how temperature affects the interaction between Spike and ACE2 by using biolayer interferometry, flow cytometry and virus capture assay. The associations between these parameters and the viral growth rate of each Omicron subvariant in the population between October 2022 and August 2023 was determined.

Results: Compared to the early D614G strain, most Omicron subvariants Spike glycoproteins possess improved ACE2 binding, enhanced immune escape and are more susceptible to cold inactivation. Their Spikes bound ACE2 in a temperature-dependent manner, enhancing Spike binding and promoting cooperativity to ACE2. We also found that Omicron subvariants Spike processing is associated with their susceptibility to cold inactivation (r = -0.6124, p = 0.0199) and with ACE2 interaction at low temperatures at the surface of pseudoviral particles (r = -0.6271, p = 0.0164). Intriguingly, we found that Spike-ACE2 binding at low temperatures is significantly associated with growth rates of Omicron subvariants in the human population (r = 0.9842, p < 0.0001).

Conclusion: Our findings indicate that Omicron subvariants acquired mutations enhancing resistance to neutralization by plasma, improving Spike processing, and increasing affinity for ACE2 at both low and high temperatures. Importantly, we found that Spike-ACE2 interaction at the surface of viral particles at low temperatures is strongly associated with Omicron subvariants growth rates. Our study underscores the necessity for ongoing surveillance of emerging subvariants and underscores the importance of measuring Spike-ACE2 interaction at low temperatures, since this parameter is highly associated with viral transmission.

147 IFNα2 Autoantibodies Post-SARS-CoV-2 Wave 1 in India Are Associated With Lower Omicron Symptomology

Enrico Bravo1, Marianne Perera2, Yasista Adiga3, Nirutha Chetan4, Asma Ahmed5, Hima Bindu6, Kaite Doore4, Adrian Hayday7, Annapurna Vyakarnam8, Stuart J. Neil9

1King’s College London, London, UK, 2John’s Research Institute, Bangalore, India

Background: Neutralizing autoantibodies against type I IFNs have been associated with life-threatening SARS-CoV-2 infection in the first wave of the pandemic. As part of a wider immunophenotyping study aimed at comparing susceptibility and immunopathogenesis of COVID-19 between the UK and India, we examined the levels of anti-IFNα2 neutralization in sera collected.

Methods: We used sera from these cohorts to measure anti-Spike (S) and anti-Nucleocapsid (N) responses, screen for IFNα2 autoantibodies that neutralized both activation of JAK-STAT signalling in a HEK-Blue IFNα beta reporter cell line, and to attenuate the antiviral state in IFNα2-treated U87-MG cells when challenged with VSV-G pseudotyped HIV-1 vectors. As controls we used autoimmune polyendocrine syndrome type 1 (APS-1) patients with a deleterious variant of AIRE, a mediator of immune tolerance leading to excessive production of type I interferon autoantibodies that ameliorate autoimmune inflammation.

Results: Most pre-pandemic individuals in both the UK and India had no detectable neutralising IFNα2 autoantibodies. In line with previous studies, a small number of UK patients (2) hospitalized during wave 1 of the pandemic had detectable IFNα2 neutralizing activity. By contrast, the majority of Anti-S/ Anti-N+ healthy donors in India had low-level neutralizing activity compared to the UK cohort. Intriguingly, amongst those hospitalized during the Omicron BA.2 wave in Bangalore, circulating anti-IFNα2 activity was significantly reduced in severe vs moderate disease. This correlated with a global increase in inflammatory phenotype in circulating leukocyte subsets in these. Notably, autoantibody levels did not correlate with age and gender across the various groups. In India, the sex distribution is closely balanced, while the UK cohorts are predominately male.

Conclusion: In the final wave of the pandemic, while high levels of autoantibodies against IFNs were associated with severe disease in previously non-exposed individuals, our results suggest the interplay between such antibodies and SARS-CoV-2 pathogenesis may be more complex in different populations. We do not understand what biological and environmental factors underlie the presence of low-level autoantibodies to IFNα2 in healthy SARS-CoV2 Ag+ individuals post first wave in India, but their reduction in severe Omicron cases suggests that their presence in conjunction with pre-existing
148 Treatment of Prehypertension in People Living With HIV: A Randomized Controlled Trial

Lily D. Yan1, Vanessa Rosziere2, Rodney Sufia3, Colette Gueteau1,4, Milraine Jean5, Fabiola Prevail1, Joseph Inddy1, Pierre Obed Fleurijean2, Alexandra Apollon2, Nour Moura2, Myung Hee Lee2, Suzanne Opafiril1, Marie Deschamps2, Jean W. Pape1, Margaret McNair1
1Wool Center Medicine, New York, NY, USA, 2GHESKIO, Port-au-Prince, Haiti, 3University of Alabama at Birmingham, Birmingham, AL, USA

**Background:** Elevated systolic blood pressure (SBP) >120 mmHg is associated with increased cardiovascular disease (CVD) risk and mortality among people living with HIV (PLWH). The dual burden of HIV and CVD is highest in low-middle income countries (LMIC), yet the World Health Organization recommends PLWH initiate medication at SBP/DBP ≥140/90 mmHg, despite lower thresholds for diabetes and renal disease. We conducted a randomized controlled trial to evaluate acceptability and mean change in SBP among PLWH with prehypertension who initiate first-line antihypertensive treatment in a LMIC.

**Methods:** A total of 250 PLWH were enrolled from GHESKIO’s HIV Clinic, between March 2021 to April 2023 in Port-au-Prince, Haiti. Participants were 18-65 years old, on stable antiretroviral therapy ≥6 months, had prehypertension (SBP 120-139 mmHg or DBP 80-89 mmHg), not on antihypertensive treatment, and randomized to intervention (initiation of amlodipine 5mg) or control (no medication unless reached SBP/DBP ≥140/90) in a 1:1 ratio. Participants were followed for 12 months with standardized clinic and community visits measuring CVD health behaviors, BPs, physical exam, imaging, and laboratory data. The primary outcome was difference in mean change in SBP between study arms, from enrollment to 12 months. Secondary outcomes were difference in mean change in DBP, acceptability, incident hypertension, and adverse events. We analyzed the primary outcome using a linear mixed-effects model accounting for repeated measures and correlations within subjects.

**Results:** The baseline characteristics of the two groups were similar. Mean SBP/DBP change over 12 months was -10.6/-8.9 mmHg in intervention and -4.6/-3.2 mmHg in control. The difference in mean change in BP between intervention vs control was SBP -5.8 mmHg (95%CI -8.77, -3.01), DBP -5.5 mmHg (95%CI -7.92, -3.16). For incident hypertension, the hazard ratio of intervention vs control was 0.43 (95%CI 0.26, 0.70). The most common adverse events (26 total) were dizziness (13) and edema (5), and no serious adverse events were drug related. Participants and study staff reported high acceptability ofamlodipine initiation.

**Conclusion:** Treatment of prehypertension in PLWH compared to standard of care reduced BP and incident hypertension, with few adverse events. There is an urgent need for CVD prevention among PLWH with elevated BP, who have alarmingly high risk of CVD events and mortality. (ClinicalTrials.gov number, NCT04692467).

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149 A Nurse-Led Strategy Improves Blood Pressure and Cholesterol in People With HIV: The EXTRA-CVD Trial

Chris T. Longenecker1, Kelley A. Jones1, Cortlynn D. Hileman2, Iwora Lance Okeke1,2, Barbara M. Gispower1, Angela Afrah3, Gerald S. Bloomfield4, Charles Muiruri2, Valerie A. Smith1, Rajesh Vedantham5, Allison R. Webel6, Hayden B. Bosworth7
1University of Washington, Seattle, WA, USA, 2Duke University, Durham, NC, USA, 3MetroHealth Medical Center, Cleveland, OH, USA, 4University Hospitals Cleveland Medical Center, Cleveland, OH, USA, 5New York University, New York, NY, USA

**Background:** Despite higher atherosclerotic cardiovascular disease (ASCVD) risk, people with HIV (PWH) experience unique barriers to ASCVD prevention care. Using a human-centered design approach, we developed EXTRA-CVD-a nurse-led multicomponent strategy of care coordination, home blood pressure monitoring, evidence-based treatment algorithms, and electronic health records tools to improve blood pressure and cholesterol management in 3 HIV clinics in the United States.

**Methods:** We conducted a randomized controlled trial among 298 PWH with suppressed HIV-1 viral load on antiretroviral therapy with comorbid hypertension and high cholesterol. Participants were stratified by site and randomized 1:1 to the EXTRA-CVD strategy or general health education control. Change in systolic blood pressure (SBP) was the primary outcome assessed at baseline, 4, 8, and 12 months. Change in non-HDL cholesterol was secondary. Primary intention-to-treat analyses were conducted using linear mixed models, with pre-specified moderation analyses by natal sex, baseline ASCVD risk, and site.

**Results:** Mean (SD) age was 58(6) years; 21% were female and 66% were non-white race. Baseline mean (SD) SBP was 135(19) mmHg and non-HDL cholesterol was 140(45) mg/dL. Half were currently prescribed 2 or more antihypertensive drugs and two-thirds were on a statin at baseline. At 12 months, participants assigned to EXTRA-CVD had 4.2mmHg (95% CI 0.3-8.2; p=0.04) lower SBP and 16.9mg/dL (95% CI 8.6-25.2; p<0.001) lower non-HDL compared to controls (Figure). Non-HDL change was driven more by a 29.5mg/dL reduction in triglycerides (95% CI 5.3-53.7; p=0.02), rather than LDL (9.6 mg/dL; 95% CI -6.3-23.5; p=0.24). EXTRA-CVD participants had higher odds of reaching treatment goal for SBP (<130/80 mmHg; OR 2.9(95% CI 1.0-8.3; p=0.05) and for non-HDL (<100mg/dL for high-risk and <130mg/dL for others; OR 7.3(2.3-23.3; p=0.001)). There was some evidence that the SBP effect was greater in females compared to males (11.8 mmHg greater at 4-months; 9.6 mmHg at 8-months, and 5.9 mmHg at 12-months; overall joint test p=0.06), but other intervention effects were similar by sex (all p>0.3). Intervention effects were not moderated by baseline ASCVD risk or site (all p>0.2).

**Conclusion:** A nurse-led multi-component strategy lowered blood pressure and cholesterol over 12 months in diverse PWH with these comorbid ASCVD risk factors. These results should inform future implementation of multifaceted ASCVD prevention programs for PWH in the United States.

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150 Community Health Worker-Facilitated Telehealth for Severe Hypertension Care in Kenya and Uganda

Matt Hickey1, Asipiah Owarganise1, Sabina Oagachi2, Norton M. Sang3, Erick Wafula Mugoma4, James Ayiek5, Jane Kabami1, Gabriel Chamie2, Elijah Rakonde1, Maya L. Petersen1, Laura B. Balzer1, Diane Haviri1, Moses R. Kampa1
1University of California San Francisco, San Francisco, CA, USA, 2Infectious Diseases Research Collaboration, Kampala, Uganda, 3Kenya Medical Research Institute, Nairobi, Kenya, 4University of California Berkeley, Berkeley, CA, USA, 5Makerere University College of Health Sciences, Kampala, Uganda

**Background:** Expanding the HIV care model to include HIV status-neutral hypertension treatment can improve cardiovascular disease outcomes; however, individuals with severe hypertension face additional barriers to care, including need for frequent clinic visits to titrate medications. We conducted a pilot study to test whether a clinician-driven, community health worker (CHW) facilitated
telehealth intervention would improve hypertension control among adults with severe hypertension in rural Uganda and Kenya.

**Methods:** We conducted a randomized controlled trial of hypertension treatment delivered via telehealth by a clinician (adherence assessment, counseling, decision-making) and facilitated by a CHW in the participant’s home, compared to clinic-based hypertension care (NCT04810650). We recruited adults ≥40 years with BP ≥160/100 mmHg at household screening by CHWs, with no restrictions by HIV status. After initial evaluation at the clinic, participants were randomized to telehealth or clinic-based hypertension follow-up. All participants were treated using standard country guideline-based antihypertensive drugs. The primary outcome was hypertension control at 24 weeks (BP <140/90); secondary outcomes included severe hypertension (BP ≥160/100) and retention in care (not late by ≥30 days at 24 weeks). We used TME to compare outcomes by arm, overall and among key subgroups.

**Results:** We screened 2,965 adults ≥40 years, identifying 266 (9%) with severe hypertension and enrolling 200 (102 control, 98 intervention). Participants were 70% women, median age 62 (IQR 51-72); 14% were HIV- positive. Mean number of hypertension drugs prescribed at last visit was 1.6 in intervention and 1.7 in control. Week 24 hypertension control was 77% in intervention and 52% in control (RR 1.48, 95%CI 1.20-1.83); effect on hypertension control was greater among women (81% vs 53%; RR 1.53, 95%CI 1.21-1.94). Prevalence of severe hypertension at 24 weeks was 7% in intervention and 25% in control (RR 0.30, 95%CI 0.14-0.64), with similar effects among people with HIV (8% vs 21%; RR 0.39, 95%CI 0.04-3.82). Retention in care at 24 weeks was 91% in intervention and 61% in control (RR 1.49, 95%CI 1.26-1.76).

**Conclusion:** Clinician-driven, CHW-facilitated telehealth for hypertension management improved hypertension control and reduced severe hypertension compared to clinic-based care. Telehealth focused on individuals with severe hypertension is a high-yield approach to improve outcomes among those with highest risk for CVD.

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**151 Pitavastatin Reduces Non-Calcified Plaque via Pro-Collagen PCOLCE Independently of LDL in REPRIEVE**

Marón Kolossváry1, Samuel R. Schmittmann2, Markella Zanni3, Kathleen V. Fitch1, Carl J. Fichtenbaum1, Judith A. Aberc4, Gerald S. Bloomfield5, Judith S. Currier6, Marissa Diggins7, Chris deFilippi8, Sara McCullam9, Michael T. Hu9, Heather J. Ribaudo9, Pamela S. Douglas9, Steven Grinspoon10

1Massachusetts General Hospital, Boston, MA, USA, 2University of Cincinnati, Cincinnati, OH, USA, 3Icahn School of Medicine at Mt Sinai, New York, NY, USA, 4Duke University, Durham, NC, USA, 5University of California Los Angeles, Los Angeles, CA, USA, 6Inova Shaw Health and Vascular Hospital, Falls Church, VA, USA, 7Harvard T H Chan School of Public Health, Boston, MA, USA, 8Duke University School of Medicine, Durham, NC, USA

**Background:** Pitavastatin reduced major adverse cardiac events (MACE) and non-calcified coronary artery plaque volume (NCPvol) among people with HIV (PWH) in REPRIEVE. However, the biological pathways responsible are not well understood. We utilized a targeted discovery proteomics approach to evaluate the biological pathways mediating statin effects on NCPvol in REPRIEVE.

**Methods:** Changes in 255 plasma protein levels (Olink, see Figure 1) were analyzed among REPRIEVE mechanistic substudy participants continuing their assigned treatment over a 2-year follow-up period. Changes in protein levels were related to changes in NCPvol in mediation analysis among participants with evidence of plaque on baseline coronary CT angiography using linear regression analysis.

**Results:** Among the 342 individuals (age: 51 years, 18% female) included in the assessment of protein changes, 275 received placebo and 267 pitavastatin. After correcting for false discovery rates, pitavastatin use was significantly associated with increased expression of 3 proteins (PCOLCE, NRP-1, MIC-A/B) and decreased expression of 4 proteins (TPPI, TRAIL, ANGPTL3, MBL2, Figure 1). Among the 196 participants (107 pitavastatin, 89 placebo) with plaque at entry, while pitavastatin associated changes in LDL were observed, they did not correlate with NCPvol (p = 0.08, p = 0.20). However, among the proteins changing with pitavastatin, the increase in PCOLCE was significantly related to the reduction in NCPvol (p = 0.27, p < 0.001). Mediation analysis including PCOLCE and LDL indicated that pitavastatin resulted in a 26% (CI: 16; 36, p < 0.001) increase in PCOLCE and a 30% (CI: 23; 37, p < 0.001) decrease in LDL. While each fold increase in PCOLCE was associated with a 25% decrease in NCPvol (CI: 13; 35%, p < 0.001), LDL changes had no relationship (2%, CI: -12; 18%, p = 0.82). Overall, 93% of the 8.8% reduction in NCPvol was mediated through the effects of pitavastatin on PCOLCE. Other proteins were either borderline or nonsignificant in the mediation analysis.

**Conclusion:** The effects of pitavastatin to reduce NCPvol in REPRIEVE were significantly mediated by changes in PCOLCE, the rate-limiting enzyme in collagen deposition. Surprisingly, LDL change was not related to changes in NCPvol. Further studies will investigate if higher levels of PCOLCE mediates the beneficial effects of pitavastatin on MACE. Statin effects on collagen formation to stabilize noncalcified plaque may be an important unrecognized mechanism to reduce coronary artery disease in PWH.

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**152 Pitavastatin Has No Effect on Long-Term, Objective Physical Function in PREPARE**

Kristine M. Erdlandson1, Trin Umblesia1, Heather J. Ribaudo1, Jennifer A. Schrack1, Edgar T. Overton1, Carl J. Fichtenbaum1, Kathleen V. Fitch2, Kenneth Wood3, Markella Zanni4, Gerald S. Bloomfield5, Pamela S. Douglas6, Steven Grinspoon7, Todd T. Brown8

1University of Colorado Arvonsatz Medical Campus, Aurora, CO, USA, 2Harvard T H Chan School of Public Health, Boston, MA, USA, 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 4University of Alabama at Birmingham, Birmingham, AL, USA, 5University of Cincinnati, Cincinnati, OH, USA, 6Massachusetts General Hospital, Boston, MA, USA, 7Frontier Science & Technology Research Foundation, Inc, Amherst, NY, USA, 8Duke University, Durham, NC, USA

**Background:** Declines in physical function occur with age, and are common among people with HIV (PWH). Due in part to the anti-inflammatory effects, statins may alleviate declines in physical function through most studies assessing the effect of statins on physical function in the general population have been observational and randomized controlled data have been limited to one year of follow-up. We hypothesized that physical function would decline among PWH, but with the known anti-inflammatory effects of statins, PWH randomized to pitavastatin would have slower declines compared to placebo.

**Methods:** PREPARE is a double-blind randomized trial evaluating pitavastatin for primary prevention of major adverse cardiovascular events in PWH; the PREPARE substudy assessed physical function in a subset of participants annually for up to 5 years. Chair rise rate based on time to complete 10 chair rises (primary outcome), 4-meter gait speed, grip strength, and a combined modified short physical performance test were analyzed using linear mixed models.

**Results:** Of 602 PWH, 52% were randomized to pitavastatin and 48% to placebo. Median age was 51 years; 18% were natal female; 2% transgender; 40% Black, and 18% Hispanic; median BMI was 27.2 (Q1 23.4, 30.1) kg/m². 45% of participants enrolled at PREPARE entry; 55% enrolled within 24 months after PREPARE treatment initiation. Median PREPARE follow-up was 4.7 (4.3, 5.0) years; 81% completed follow-up. Physical function was similar between the two treatment groups at PREPARE entry. There was no evidence of decline in chair rise rate in either treatment group (Figure), and no significant difference in the pitavastatin group compared to placebo (difference -0.10 (95% CI -0.30, 0.10) rises/min/year; p = 0.31). Small declines were observed in other physical function tests in both treatment groups, with no apparent differences between groups (Figure). The findings were consistent in subgroup analyses by age, sex, race, ART duration, CD4 cell count, baseline physical function, and presence of muscle symptoms (not shown).

**Conclusion:** We observed minimal declines in physical function over 5 years of follow-up among middle-aged PWH, with no differences among PWH randomized to pitavastatin compared to placebo. Our findings do not support
the use of statins to maintain physical function in this population, but do expand upon the overall REPRIEVE trial findings to support the long-term safety of statin therapy on physical function in PWH.

### Lung Function, HIV and Mortality: Analyses From the AIDS Linked to the Lung Health in PWH

Background: Chronic lung disease is an increasingly important comorbidity for persons aging with HIV. Persons with HIV/PWH can experience accelerated decline in lung function, including spirometry measures (FEV1). The current implications of this lung function decline on all cause and HIV-related mortality warrants further investigation. Additionally, the normalized FEV1Q has recently been developed to improve interpretation of lung function without biases related to sex or race but has not yet been studied in cohorts of PWH. We leveraged the AIDS Linked to the Invasive Experience (ALIVE) cohort in Baltimore, MD, consisting of PWH and matched HIV-uninfected participants, to study the implications of impaired lung function in a high-risk HIV cohort.

Methods: We analyzed 2009-2019 ALIVE participant data. Lung function (FEV1 and FEV1Q) and clinical data (HIV RNA, comorbidity data) were collected at semi-annual visits. Mortality was derived from the national death index and clinical records, assessing all-cause and mortality due to HIV and chronic disease. Associations between time-updated lung function and mortality were analyzed using Cox proportional hazard models- including observation-for appropriate patient groups.

Results: 1534 participants (474 PWH) contributed 10515 lung function measures over 10 years. Mean age at entry was 50, with 34% reporting active IDU and 84% current smokers. Among PWH, 53% had detectable HIV RNA. Mortality was high with 410 (26%) deaths during the study period; 35% among PWH. In adjusted models, accounting for comorbidities and risk factors, a 1SD increase in FEV1 (HR 0.70; P<0.01) and FEV1Q (HR 0.73; P<0.01) was associated with lower all-cause mortality. Higher lung function was protective for HIV-related mortality (FEV1; HR 0.52, P<0.01; FEV1Q, HR 0.57, P<0.01). Among PWH, after adjusting for viremic control, lung function remained associated with all cause (HR 0.62, P<0.01) and HIV-related mortality (HR 0.54; P<0.01). Conclusion: We demonstrate associations in a high-risk HIV cohort, after accounting for behavioral risk factors and comorbidities, lung function remains highly associated with all cause and HIV related mortality. These results are consistent when using the normalized FEV1Q, which may be less susceptible to bias. The results highlight the importance of lung disease and interventions to preserve lung health in PWH.

### Prostate Cancer Characteristics and Outcomes for Veterans With HIV in the Antiretroviral Era

Background: Prostate cancer is the leading cancer diagnosis among Veterans with HIV and will soon be the leading cancer among all US persons with HIV (PWH). Despite the substantial prostate cancer burden for PWH, there are little data on prostate cancer clinical characteristics and outcomes. Therefore, we studied prostate cancer characteristics at diagnosis and survival by HIV status in the Veterans Aging Cohort Study (VACS)-HIV, a national cohort of Veterans with HIV and demographically similar Veterans without HIV.

Methods: We used data from VACS-HIV (2001-2018) to identify a cohort of male PWH prior to prostate cancer diagnosis (n=791), as well as male comparators without HIV (PWoH n=2,778). We compared patient demographics, prostate-specific antigen (PSA) testing and prostate cancer clinical characteristics by HIV status. We then compared prostate cancer risk groupings (D’Amico) and prostate cancer-specific and overall survival by HIV status, stratified by risk group using age-adjusted Cox regression models.

Results: VACS-HIV patients with prostate cancer had a median age of 62 years, which did not differ by HIV status. Race/ethnicity proportions were also similar, with non-Hispanic Blacks being the most common group diagnosed with prostate cancer. PWH with prostate cancer frequently had detectable HIV viremia at prostate cancer diagnosis (>60%). HIV infection was associated with higher PSA (median 6.8 vs. 6.3 ng/mL; p=0.005) but no difference in Gleason grade. There was less frequent PSA testing among PWH prior to prostate cancer diagnosis (1.25 fewer tests than PWoH, age adjusted; p<0.001). PWH were more likely to be diagnosed with D’Amico intermediate/high risk localized prostate cancer (68% vs. 63%; p=0.02) and advanced prostate cancer (either nodal involvement or metastatic disease) than PWoH (4.0% vs. 2.7%; p=0.04). Both relationships persisted after adjustment for age. HIV was significantly associated with worse age-adjusted all-cause mortality for intermediate-, high-risk localized and advanced cancers. PWH did not have higher prostate-cancer specific mortality in any cancer risk group compared to PWoH.

Conclusions: PWH were diagnosed with higher risk prostate cancers more frequently in VACS-HIV than those without HIV possibly reflecting lower rates of PSA testing in this group. Higher non-cancer mortality seen in those with HIV infection may impact the relative risks and benefits of prostate cancer management strategies- including observation-for appropriate patient groups.
InSTI Switch During Menopause Is Associated With Accelerated Body Composition Change

Rebecca Abelman1, Yifei Ma1, Cyra C. Mehta2, Qian Yang3, James Brack1, Maria L. Alcâide1, Anjali Sharma1, Michelle Floris-Moore1, Elizabeth F. Topper1, Kathleen Weber1, Sebile Kassaye2, Deborah Gustafson2, Leslie D. Lahiri3, Phyllis Tien1

1University of California San Francisco, San Francisco, CA, USA, 2Emory University, Atlanta, GA, USA, 3University of Miami Medical Center, Jackson, FL, USA, 4University of Miami, Miami, FL, USA, 5Albert Einstein College of Medicine, Bronx, NY, USA, 6University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 7The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 8Cook County Health & Hospitals System, Chicago, IL, USA, 9Georgetown University, Washington, DC, USA, 10State University of New York Downstate Medical Center Downstate Medical Center, Brooklyn, NY, USA

Background: Integrase strand transfer inhibitors (INSTIs) have been associated with greater weight gain in women with HIV (WWHO) than men with HIV. The transition to menopause is also associated with body composition changes. Whether INSti initiation during the menopausal transition affects waist circumference (WC) and BMI trajectories is unknown.

Methods: From 2006-2019, 1159 non-pregnant virally-suppressed WWHO (424 who switched to an INSTI (INSTI+); 735 who did not switch (INSTI–) during a similar time window) and 904 women without HIV (WWHO) from the Women's Interagency HIV Study were included. The visit at which WWHO reported switching to an INSTI was defined as the index visit. Mixed effect models including quadratic terms were used to evaluate change in WC and BMI by menopausal phase defined using anti-Müllerian hormone, a biomarker of ovarian reserve, at the index. Models were adjusted for demographics, baseline WC or BMI, behavioral factors, comorbidities, and HIV-related factors.

Results: Overall, 66% identified as Black and 28% were premenopausal, 10% early peri-, 28% late peri-, and 34% postmenopausal at the index. INSTI+ were older than INSTI– and WWHO (median 52 vs 49 vs 47 years) with median BMI 30 vs 29 vs 31.5 kg/m², respectively. 64% of INSTI+ and 79% of INSTI– were on tenofovir DF prior to the index visit. Figure shows the WC trajectory for INSTI+, INSTI–, and WWHO who were premenopausal and late perimenopausal at index visit. In premenopausal women, INSTI+ and INSTI– was associated with a 0.06 cm per 6 mos (95% CI: -0.26, 0.08) and 0.08 cm per 6 mos (95% CI: -0.28, 0.00) faster increase than INSTI–. In late perimenopausal women, when compared to WWHO, INSTI+ was associated with significantly faster increases in WC which peaked at 41 mos and then declined, while INSTI– had smaller increases in WC (0.14 cm per 6 mos; 95% CI: 0.55, 0.33; INSTI+ had a 0.39 cm per 6 mos (95% CI: 0.15, 0.63) faster linear increase in WC than INSTI–. In postmenopausal women, INSTI+ was associated with faster WC increases up to 39 mos then declines compared to INSTI–. BMI trajectories were similar for peri- and postmenopausal women.

Conclusion: Switching to an INSTI-based regimen during late peri- and postmenopause is associated with early accelerated increases in WC and BMI when compared to women who did not switch. Our findings suggest that menopausal status should be considered when switching to an INSTI.

Simultaneous Initiation in ART-Naive PWH of DTG-Based ART & 3HP Maintains Efficacious DTG Levels

EtheL D. We1, Belén Perez Solanas,2 Iasadora Salles1, Bareng Alette Nonyane1, M Sebe1, Trevor Beattie1, Manasa Mapendere1, Tanya Nielsen1, Jayajothi Moodley1, Violet Chihota1, Rada Savic1, Kelly E. Dooley1, Richard E. Chaisson2, Savin Churchyard1, for the UNITAID IMPAACT4TB Consortium

1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2University of California San Francisco, San Francisco, CA, USA, 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 4The Aurum Institute, Johannesburg, South Africa, 5Vanderbilt University, Nashville, TN, USA

Background: People with HIV (PWH) living in areas of high TB endemicity have high potential to benefit from short-course tuberculosis (TB) preventive therapy (TPT) with weekly isoniazid and rifapentine for 3 months (3HP). 3HP given to virally suppressed PWH on dolutegravir (DTG)-based regimens has been found to be safe and to maintain virologic suppression with adequate DTG levels. No data yet supports simultaneous 3HP with DTG-based ART initiation among ART-naive people.

Methods: A phase 1/2 comparative trial of simultaneous initiation of 3HP or 6 months isoniazid (6H) with DTG-based ART was performed among antiretroviral (ART)-naive adults with HIV in South Africa. Participants were sequentially enrolled and assigned to 6H (N=25) and 3HP (N=50). PK sampling for DTG trough was performed at: day 1 (prior to first HP dose), day 17 (3 days after 3rd HP dose), and day 52 (3 days after 8th HP dose) in both groups. Primary endpoints were safety and DTG pharmacokinetics during 3HP/DTG coadministration. Safety and the secondary endpoint of HIV viral load at TPT completion were previously reported.

Results: By week 8, viral suppression (VL < 50 copies/mL) was achieved in 100% of participants in both groups. At week 12, viral suppression (VL < 50 copies/mL) was present in 44/50 (88%) and 23/25 (92%) participants in the 3HP and 6H groups, respectively. Nonlinear mixed-effects modeling showed that there was a 72% induction effect of rifapentine on DTG clearance, from 0.95 L/hr to 1.64 L/hr. DTG trough concentration was above the FA-Ic50 (64 ng/mL) in all participants at all timepoints, and >158 ng/mL (the lower 5th percentile confidence bound of concentrations achieved with fully suppressive 10 mg daily dosing in the SPRING-1 trial) in all but 2 individuals at week 3 and in all individuals at week 8. The 2 individuals w DTG concentrations below 158 ng/mL at week 3 both suppressed by week 8.

Conclusion: Simultaneous initiation of 3HP for LTBI treatment and DTG-based ART resulted in rapid viral suppression among ART-naive PWH. Despite a 72% induction of DTG clearance in the 3HP group, DTG concentrations enabled viral suppression < 50 copies/mL in all individuals in both groups at 8 weeks. This information is critical to informing global guidelines around LTBI treatment in ART-naive individuals.
**Early Bacterial Activity of the Alpibectir-Ethionamide (AlpE) Combination Against Tuberculosis**

Jeanette DePrez1, Ceyrin Upton1, Laurynas Mockeliusa, Michel Pieren, Ulrika Simonsson, Andreas H. Diacomi, Glenn E. Dale1, Pierre Delige1, Lisa Husband, Simon Tiberi, Sophie Pemman, Thabo Mabuka, Mandi Nieuwenhuyzen, Anteneh Yalwé, Veronique de Jager

1TASk Applied Science, Cape Town, South Africa; 2Uppsala University, Uppsala, Sweden; 3Bioversys AG, Basel, Switzerland; 4Bioversys SAS, Lille, France; 5GlaxoSmithKline, London, UK

**Background:** Dose-dependent tolerability of the utility of ethionamide for tuberculosis (TB) treatment at standard doses (750 to 1000 mg). Alpibectir (formerly BVL-GSK098) stimulates an alternative pathway of bacterial ethionamide bioactivation leading to retained activity at lower exposures in vivo. We report results on the first cohort evaluating the early bacterial activity (EBA), safety and tolerability of the alpibectir-ethionamide (AlpE) combination (NCT05473195).

**Methods:** Adults with newly diagnosed, rifampicin- and isoniazid-susceptible pulmonary TB were randomised 5:1 to receive 7 days of AlpE 9 mg/250 mg or isoniazid 300 mg as microbiological control. EBA was assessed by the change in time to culture positivity (TTP-EB)–7 using a mixed-effects model. Serum concentrations of ethionamide and its active sulfoxide metabolite were explored as covaries in a pharmacokinetic/pharmacodynamic (PK/PD) model. Treatment-emergent adverse events (TEAEs) were assessed daily.

**Results:** 15 participants were randomised to AlpE and 3 to isoniazid. Most participants were male (78%) with a mean age and weight of 33.6 years and 52.9 kg, respectively. One participant withdrew for reasons unrelated to treatment. Median TTP-EB–7 (2.5th–97.5th percentiles) was 45.28 (28.78–78.12) and 48.41 (42.02–54.89) hours for AlpE and isoniazid, respectively. Isoniazid activity was in range of previous results. The median maximum serum concentrations (C) of ethionamide (1230 ng/mL) and ethionamide-sulfoxide (2050 ng/mL) were reached approximately 1 hour after AlpE administration. The mean half-life for ethionamide and ethionamide-sulfoxide was 1.47 hours. Median area under concentration-time curve (AUC) of ethionamide and ethionamide-sulfoxide was 3662 and 5119 h*ng/mL, respectively. Higher ethionamide-sulfoxide exposure significantly increased EBA where each 100 h*ng/mL unit of increase resulted in a 3.68% increase in time to positivity (TTP) slope. 19 (73%) of the 26 mild (76.9%) and moderate (23.1%) TEAEs occurred within the first 28 days of treatment. Median TTP-EBA0–7 (2.5th–97.5th percentiles) was 45.28 (28.78–78.12) and 48.41 (42.02–54.89) hours for AlpE and isoniazid, respectively.

**Conclusion:** AlpE was well tolerated, safe and showed bacterial activity similar to isoniazid in participants with tuberculosis. AlpE can be added to the growing list of novel antituberculosis agents for drug-susceptible and drug-resistant TB. The study is ongoing with escalating doses of alpibectir and ethionamide to optimize the combination.

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**NAFLD and Advanced Fibrosis Are Common in Adults With HIV and Associated With Unique History**


1University of Texas at Houston, Houston, TX, USA; 2Cleveland Clinic, Cleveland, OH, USA; 3Indiana University, Indianapolis, IN, USA; 4University of California San Francisco, San Francisco, CA, USA; 5Duke University, Durham, NC, USA; 6The Johns Hopkins University, Baltimore, MD, USA; 7University of Alabama at Birmingham, Birmingham, AL, USA; 8Virginia Commonwealth University, Richmond, VA, USA; 9University of California San Diego, La Jolla, CA, USA; 10The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

**Background:** Non-alcoholic fatty liver disease (NAFLD) may be more common among people with HIV (PWH), but unique risk factors for NAFLD in PWH (NAFLD-PWH) are poorly understood. We examined the prevalence of and risk factors for NAFLD and advanced fibrosis (AF) in a cohort of PWH without other known causes of liver disease, as well as histological features of NAFLD in persons with and without HIV.

**Methods:** In an ongoing prospective study, PWH ≥ 18 years of age on suppressive antiretroviral therapy (ART) were screened for NAFLD (controlled attenuation parameter ≥ 263 dB/m) and AF (liver stiffness measurement ≥ 11 kPa) using vibration controlled transient elastography. For histology, 107 biopsies each from NAFLD-PWH (cases) and NAFLD in people without HIV (controls) were matched on age/sex/race/ethnicity/BMI/ALT. Biopsies were centrally read using the NASH CRN scoring system. Logistic regression evaluated associations with NAFLD and AF.

**Results:** PWH (n = 654) had mean age 53 years, 73% male sex at birth, 5% were non-Hispanic Black and 20% Hispanic. NAFLD and AF prevalence were 53% and 6%, respectively. Older age, male sex, greater BMI or waist circumference and higher ALT and triglyceride concentrations associated with greater NAFLD odds, and non-Hispanic Black race with lower odds. Greater BMI or waist circumference, higher AST and alkaline phosphatase concentrations and lower platelet counts associated with greater odds of AF, and non-Hispanic Black race with lower odds (all p < 0.05). NAFLD-PWH had less steatosis (63% grade 1/2 vs. 47%, p = 0.01), less inflammation (70% grade 1/2 vs. 60%, p = 0.03) and less hepatocyte ballooning (6% vs. 45%, many 15% vs 27%, p = 0.03) and portal inflammation (8% vs. 27%) than controls. As a result, NAS was lower in NAFLD-PWH (3.2 ± 1.6 vs. 4.0 ± 1.6, p < 0.001), with a trend towards less steatohepatitis (61% vs. 71%, p = 0.09). Regression analyses observed less steatosis, portal inflammation and ballooning but more fibrosis in NAFLD-PWH (all p < 0.03).

**Conclusion:** In summary, in our cohort of PWH undergoing systematic screening, NAFLD prevalence was high, with traditional metabolic risk factors but not HIV-ART-specific characteristics dominating risk for NAFLD and AF. Traditional histologic drivers of fibrosis were less pronounced in NAFLD-PWH and yet fibrosis stage was higher vs. matched controls without HIV, suggesting HIV-specific factors beyond hepatic necroinflammation may contribute to fibrosis in NAFLD-PWH.

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**Semaglutide Reduces Metabolic-Associated Steatotic Liver Disease in People With HIV: The SLIM LIVER**

Jordan E. Lake, Douglas W. Kitch, Amy Kantor, Raja Mathupillai, Karin Klingman, Christina Vernon, Carl I. Fichtenbaum, Sonya Heath, Hugo Perazzolo, Kathleen Carey, Todd T. Brown, Alan Landay, Fred R. Sattler, Kristine M. Elrod, Alan University of Texas at Houston, Houston, TX, USA; 2Harvard T.H. Chan School of Public Health, Boston, MA, USA; 3Forespect, PLLC, Houston, TX, USA; 4National Institutes of Health, Rockville, MD, USA; 5OHL Corporation, Silver Spring, MD, USA; 6University of Cincinnati, Cincinnati, OH, USA; 7University of Alabama at Birmingham, Birmingham, AL, USA; 8Gwadzawo Cruz Foundation - Fio-Cruz, Rio de Janeiro, Brazil; 9Massachusetts General Hospital, Boston, MA, USA; 10The Johns Hopkins University School of Medicine, Baltimore, MD, USA; 11Rush University, Chicago, IL, USA; 12University of Southern California, Los Angeles, CA, USA; 13University of Colorado Anschutz Medical Campus, Aurora, CO, USA

**Background:** Metabolic-Associated Steatotic Liver Disease (MALSID) is common among people with HIV (PWH) and likely synergistic with HIV-1 to accelerate hepatic injury and organ dysfunction. The glucagon-like peptide-1 receptor agonist semaglutide is associated with cardiometabolic improvements in the general population through its effects on weight reduction and systemic inflammation. We designed a phase IIb, single-arm, pilot study of the effects of semaglutide on magnetic resonance imaging proton density fat fraction (MRI-PDFF)-quantified intrahepatic triglyceride (IHTG) content in PWH and MALSID.
Trends in HIV and HCV Prevention Efforts and Incidence Among People Who Inject Drugs in Baltimore

Eshan Patel1, Becky Genberg2, Bryan Lau2, Jacqueline E. Rudolph2, Jaquie Astemborski1, Rachel E. Gicquel3, Danielle Germain4, David D. Celentano2, David Vlahov2, Stefanie Strawther3, Greg Kirk2, David Thomas2, Shruthee M. Mehta2

1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2University of Wisconsin–Madison, Madison, WI, USA, 3Yale University, New Haven, CT, USA, 4University of California San Diego, La Jolla, CA, USA, 5The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Advances in HIV and hepatitis C virus (HCV) prevention and treatment have led to plans to end the HIV epidemic and achieve HCV elimination by 2030. Data on long-term trends in the uptake of combination HIV/HCV prevention services and their impact on HIV/HCV incidence among people who inject drugs (PWID) are limited.

Methods: The AIDS Linked to the Intravenous Experience (ALIVE) study is a community-based cohort of PWID aged ≥18 years in Baltimore, Maryland with 5 enrollment periods: 1988-89, 1994-95, 1998, 2005-08, and 2015-18. We assessed trends in HIV and HCV seroincidence, prevalence of injection practices, self-reported use of prevention and treatment services, HIV viremia (>400 c/mL) and HCV viremia (>500 IU/mL) using Poisson and logistic regression with 5 enrollment periods: 1988-89, 1994-95, 1998, 2005-08, and 2015-18. Participants (n=49) had median age 52 years, BMI 35 kg/m², 39% Hispanic ethnicity and 33% Black/African American race; 63% were cis or trans women and 82% were on injectable estradiol replacement (art) and 9% were on injectable methadone use (10% in 1988; 22% in 2001; 44% in 2019) and syringe service programs use (36% in 1998; 44% in 2001; to 51% in 2019). Later declines in HIV incidence were also associated with increases in ART use (55% in 2006; 96% in 2019) and declines in HIV viremia (63% in 2007; 36% in 2019). However, HCV seroincidence remained high during periods in which HCV treatment uptake increased (2% in 2014; 56% in 2019) and HCV viremia declined (84% in 2006; 36% in 2019).

Conclusion: In a cohort of PWID, HIV and HCV seroincidence decreased over time corresponding with increased prevention efforts and behavioral changes; however, HCV seroincidence remained high. Intensified efforts are needed to achieve HCV elimination among PWID.

Preclinical Pharmacokinetic Assessment of a Hepatitis C Virus Long-Acting Injectable Formulation

Usman Arshad1, Henry Pertinez2, Joanne Sharp3, Joanne Herriott4, Edyta Kijak2, Eduardo Gallardo-Toledo2, Andrew B. Dwyer5, Catherine Unsworth5, Alison C. Savage5, James I. Hobson5, Lee Tatham5, David Thomas2, Paul Curley2, Steve Rannard2, Andrew Owen5

1University of Liverpool, Liverpool, UK, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Eight weeks of daily, oral glecaprevir (G) and pibrentavir (P) cures 98% of people with chronic Hepatitis C Virus (HCV) infection. However, major challenges, including medication adherence and loss of patients to follow-up, impede delivery of this combination. As such, 58 million people remain infected worldwide. A long-acting injectable (LAI) providing 8 weeks of pharmacokinetic exposure from a single administration would overcome many of these challenges by providing a one-shot cure. Moreover, G and P possess key physicochemical and pharmacokinetic properties shared by other LAI paradigms.

Methods: G and P were co-formulated as a fixed dose combination LAI (250 mg/mL G, 250 mg/mL P). Dosing volumes of 0.075, 0.15 and 0.3 mL (representing active doses of 18.75, 37.5 and 75 mg) were administered to male Sprague Dawley rats (n = 4, 250-300 g) via intramuscular injections to the thighs. Plasma samples were collected from the tail vein over 13 weeks and livers were harvested at the end of the study. G/P concentrations were quantified in plasma and liver using validated LC-MS/MS.

Results: Plasma concentration-time profiles for G and P demonstrated a dose-proportional increase in exposure (Fig. 1) with dose-linear increases in both plasma and liver concentrations. However, G/P LAI demonstrated sustained pharmacokinetic exposure from a single administration would overcome many of these challenges by providing a one-shot cure. Moreover, G and P possess key physicochemical and pharmacokinetic properties shared by other LAI paradigms.
required to optimise drug ratios and confirm safety through GLP toxicology assessments to support first-in-human evaluation.

![Figure 1. Plasma concentration profiles for (a) Gliclazepirin and (b) Pibrentasvir in Rats.](image)

### 162 Intrahepatic HDV Activity Is Fueled by Integrated HBV DNA-Derived HBs Transcripts

#### Background:
HDV exploits HBV surface proteins (HBs) for its morphogenesis and de novo entry into hepatocytes. Here, we investigate HBV and HDV replicative activity and their still undefined interplay in liver biopsies from patients (pts) with chronic co-infection.

#### Methods:
Liver tissue was analysed from 25 pts (71% NUC-treated; 96% HBeAg[-]). Intrahepatic levels of covalently closed circular DNA (cccDNA), transcripts, mainly derived from integrated HBV-DNA. These issues are crucial for defining mechanisms underlying HDV persistence, that could hamper the success of therapeutic strategies.

#### Results:

- HDV replication pathway acts independently from the size of intrahepatic HBV reservoir and is fueled by an abundant production of HBs transcripts, mainly derived from integrated HBV-DNA.
- Overall data suggest the existence of independent HBV and HDV replicative pathways.

#### Conclusion:
HDV replication pathway acts independently from the size of intrahepatic HBV reservoir and is fueled by an abundant production of HBs transcripts, mainly derived from integrated HBV-DNA. These issues are crucial for defining mechanisms underlying HDV persistence, that could hamper the success of therapeutic strategies.

#### A 4-Month Regimen of Quabodepistat, Delamanid, and Bedaquiline for Pulmonary TB: Interim Results

#### Background:
There remains an urgent need for short-duration, potent, and safe anti-tuberculosis (TB) agents effective against drug-susceptible and drug-resistant strains of Mycobacterium tuberculosis. Quabodepistat (QBS; formerly OPC-167832) is a novel anti-TB agent that targets decaprenylphosphoryl-β-D-ribose 2'-oxidase (DprE1). In a prior study, QBS in combination with delamanid (DLM) and bedaquiline (BDQ) for 14 days was well tolerated and exhibited similar early bactericidal activity to the standard of care regimen, RHEZ (rifampicin, isoniazid, ethambutol, and pyrazinamide) in patients with drug-susceptible pulmonary TB (DS-TB).

#### Methods:
This interim analysis of the phase 2b/c, randomized trial (NCT05221502) evaluates the safety, efficacy, and pharmacokinetics of QBS in combination with DLM and BDQ for 4 months in participants with DS-TB compared to 6-month RHEZ treatment. Participants were randomized (1:2:2:1) stratified by chest x-ray bilateral cavitation and HIV status) to once-daily QBS 10 mg, 30 mg, or 90 mg in combination with DLM and BDQ, or RHEZ. The follow-up period (to 52 weeks post-randomization) is ongoing. The primary endpoint was proportion of participants achieving sputum culture conversion (SCC) by the end of the treatment period. Here, we report interim results after 117/122 (96%) randomized participants completed study treatment.

#### Results:

- At enrollment, most participants were male (65%), of Black African race (68%) with a median age of 31 years (range 18–65), a median BMI of 19 kg/m², with bilateral cavitation (19%), and HIV-negative (100%) status. The modified intention-to-treat population (n=121), SCC at end of treatment was achieved by 96% (96/100) in the pooled QBS arms and 91% (19/21) in the RHEZ arm (Table 1). Similar SCC rates were observed in the per-protocol analysis (n=108). The percentages of participants experiencing at least one Division of AIDS ≥Grade 3 adverse event (AE) were 15%, 12%, 11%, and 5% in the QBS 10 mg, 30 mg, and 90 mg arms, respectively. There was one treatment discontinuation due to death (QBS 30-mg arm) from severe/worsening TB. No serious AEs were attributed to trial medications. No clinically significant QTc prolongation events, QTc prolongation ≥500 ms, or liver enzyme (ALT/AST) elevations ≥5 times the upper limit of normal were reported.

#### Conclusion:
In this interim analysis, high rates of SCC were achieved with the QBS-based three-drug treatment regimen. The regimen was generally well tolerated and warrants further investigation.
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Provisional Results From a 3-month Clofazimine/Rifapentine-Containing Regimen for Drug-Sensitive TB

John Metcalfe1, Isabelle Weir2, Kimberly K. Scarsi3, Samuel Pierre3, Austin Van Grack4, Cecilia Kanyama1, Maxwell Yohane1, Wadzani Samaneza1, Heetal Hervrekar5, Jorge Leon-Cruz6, Alberto Mendoza-Ticona7, Melanie Gott8, Richard E. Chaixson9, for the ACTG A5362 CLO-FAST Study Team

1University of California San Francisco, San Francisco, CA, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3University of Nebraska Medical Center, Omaha, NE, USA, 4Gilead, Port-au-Prince, Haiti, 5ULH Corporation, Bethesda, MD, USA, 6University of North Carolina Project–Malawi, Lilongwe, Malawi, Malawi, 7Kamuzu University of Health Sciences-Johns Hopkins Research Project, Blantyre, Malawi, 8University of Zimbabwe, Harare, Zimbabwe, 9The Johns Hopkins University-Baltimore-India Clinical Trials Unit, Pune, India, 10Socios en Salud Suramericano Peru CRS, Lima, Peru, 11National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 12The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Clo-Fast (ACTG A5362; NCT04311502) is a randomized, controlled, open-label phase IIc clinical trial evaluating the safety and efficacy of a 3-month rifapentine (P1200mg)/clofazimine (CFZ)-containing regimen versus 6-month standard of care (SOC) for drug-susceptible (DS) TB that stopped enrollment early due to lack of clinical efficacy. We present results from data through Sept 25, 2023, when the last participant completed study treatment.

Methods: Adults enrolled at six sites in Malawi, Zimbabwe, South Africa, India, and Haiti. Randomization, stratified on HIV status and advanced disease on chest X-ray, was to: Arm 1 (13-week experimental regimen): 3P (PFZE/CFZ); Arm 2 (26-week SOC): 2RHZ/4RI or Arm C (PK subgroup without CFZ load, then SOC): 1P (PFZE/HRZ/4RI). The primary outcomes were (efficacy) time to stable lung culture conversion through week 12 and (safety) proportion experiencing an AE ≥ grade 3 through week 65 in Arms 1 and 2. The proportion with an unfavorable clinical/bacteriologic outcome in Arms 1 and 2 by week 65 was a key secondary outcome.

Results: Between June 2021 and April 2023, when the trial was stopped for inefficacy per DSMB recommendation, we enrolled 58 of 110 planned participants to Arm 1, 31 of 55 planned participants to Arm 2, and 15 of 20 planned participants to Arm C. Most participants were male (79%), 29% were living with HIV (median baseline CD4+ 265 cell/mm3, IQR 185-379 cell/mm3), 71% had radiographically advanced TB disease, and 25% were ≥3 smear-positive at entry. Median (IQR) follow-up on study was 50.2 weeks (39.7, 57.6 weeks). By week 12, 89% (n=48) in Arm 1 and 90% (n=28) of participants in Arm 2 achieved stable culture conversion (adjusted HR 1.17, 90% CI 0.79 to 1.73, adjusted for baseline HIV status and presence of advanced disease). The cumulative proportion of participants experiencing an AE ≥ grade 3 was higher in Arm 1 (146%) than Arm 2 (16%; difference 30%, 90% CI 14-45%), driven by change in creatinine clearance in Arm 1. The cumulative probability of an unfavorable outcome in Arm 1 was 49% (95% CI 34-67%) versus 24% (95% CI 19-58%) among Arm 2 participants (difference -15%, 95% CI -41% to 11%). The table describes the clinical/bacteriologic unfavorable outcome events.

Conclusion: A 13-week regimen containing CFZ and rifapentine was not efficacious for treatment of DS-TB. The SOC arm also had a high rate of unfavorable events, despite high week 12 culture conversion.

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Patrick S. Sullivan, Stephanie Dubose, Kamaria Brisco, Gordon Le, Marta Juhasz

Emory University (Atlanta, GA, USA)

Background: Pre-exposure prophylaxis (PrEP) is highly effective to reduce risk of HIV infection; the population-level impact of PrEP is predicted to depend on PrEP coverage (e.g., PrEP prescriptions among those with indications), adherence to prescribed PrEP, the extent to which PrEP is used by those at greatest risk for HIV infection, and the extent of viral suppression among community risk contacts.

Methods: We used publicly available data on PrEP prescriptions and calculated PrEP coverage per 100 persons with indications in each state during each year. We calculated quintiles of mean PrEP coverage (e.g., proportion of PrEP users among those with an indication for PrEP) during 2012-2021 for 50 US states and the District of Columbia. For each quintile, we calculated the estimated annual percent change (EAPC) in HIV diagnosis rates from 2012-2021 with 95% confidence intervals using temporal trends models and calculated a p value for trend across state-specific quintiles. Because higher PrEP coverage in a state might be confounded by higher levels of viral suppression, we adjusted EAPC estimates for prior year state-level viral suppression.

Results: The estimated state-specific EAPCs in HIV diagnosis rates between 2012-2021 ranged from -11.9% (95% CI: -13.0%, -10.8% in Washington, DC) to +10.5% (95% CI: +5.1%, +16.2% in West Virginia). Mean PrEP coverage among states and the District of Columbia between 2012-2021 ranged from 3.8% (West Virginia) to 22.2% (New York). From 2012-2021, the quintile-specific change in HIV diagnosis rates ranged from a 1.7% increase (95% CI: -0.7% to +4.1%) in the lowest quintile of PrEP coverage to an 8.0% decrease (95% CI: -9.3% to -6.8%) in the highest quintile of PrEP coverage, after controlling for yearly changes in viral suppression rates (Figure; p value for trend across quintiles: 0.0077).

Conclusion: In an ecologic analysis, increasing PrEP coverage was associated with decreasing new HIV diagnoses from 2012-2021 among US states, even controlling for differences in state-viral suppression. Our data suggest that PrEP coverage is a meaningful measure to assess the progress of PrEP programs. However, our analysis also documented stark differences in the trajectories of PrEP program among US states: there was an 8-fold difference between the extent to which PrEP needs were met between the lowest and highest performing states. PrEP coverage data is useful to monitor progress in state PrEP programs.
166 HIV Incidence in Users of HIV Preexposure Prophylaxis in Australia: A Whole-of-Population Analysis
Nicholas A. Medland, Hamish McManus, Benjamin Bavinton, Michael Traeger, Doug Fraser, Andrew Grulich, Mark Stovee, Ske Maygrover, Jonathan King, Dash Heath-Patoyer, Rebecca Gay
1University of New South Wales, Sydney, Australia, 2Monash University, Melbourne, Australia
Background: Use of HIV pre-exposure prophylaxis (PrEP) at scale has been associated with reduced community HIV transmission: diagnoses of recently acquired HIV (under one year) among gay and bisexual men in Australia fell from 223 in 2018 to 107 in 2022. We examined HIV incidence and risk factors in all people receiving PrEP in Australia’s national health system.
Methods: Linked de-identified records for all government subsidised PrEP and antiretroviral therapy (ART) from April 2018 to June 2023 allowed us to identify HIV acquisition in PrEP users who initiated ART. ART initiation was used as a proxy for HIV acquisition given high rates of HIV testing among PrEP users (at least 6-monthly) and high treatment uptake (over 95% after six weeks) in Australia. The date of HIV acquisition was the midpoint between 30 days before ART initiation and either six months prior or the most recent PrEP prescription. We calculated days covered by PrEP and HIV incidence in people using PrEP and its predictors using Poisson regression over the study period of April 2018 to December 2022.
Results: Of 62,563 people receiving PrEP (97.8% men, median age 33), 190 acquired HIV during the study period with an overall incidence rate of 1.09/1000 person years (95% CI 0.94-1.25). HIV incidence was 2.65/1000 PrP users once dispersed PrEP once only (20.0% of PrEP users, 31.6% of HIV cases), compared with 1.02/1000 among those with <60% of days covered (52.4% of PrEP users, 54.2% of HIV cases) and 0.53/1000 among those with ≥60% of days covered (28% of PrEP users, 14% of HIV cases). Using the group dispersed PrEP only once as a comparator, those with ≥60% days covered had an 80.2% reduction in incidence (p<0.001) and those with <60% days covered had a 61.5% reduction (p=0.009). Incidence was also higher in specific subgroups: those with a history of HIV (10.05/1000 PrP users, 6.3% of HIV cases) and 18-29-year-olds (1.32/1000, 35.1% of PrEP users, 40.0% of HIV cases). PrEP usage, younger age and hepatitis C treatment were independent predictors of HIV incidence.
Conclusion: HIV acquisition in people previously engaged in PrEP accounted for 57.9% of diagnosed newly acquired HIV among gay and bisexual men in Australia in 2022, highlighting the need for interventions focused on this population to achieve elimination. In particular, support is needed for those who don’t return for repeat dispensing and less frequent PrEP users. Programs should also be tailored for specific socio-demographic characteristics.

167 High PrEP Uptake and Adherence Measured Objectively Among Young African Women in the INSIGHT Cohort
Brenda G. Miremba, Mieghan Krown, Zhinwe Zwide, Elizabeth Bukusi, Ravinder Panchal, Cheryl Louna, Noluthando Mwelase, Pearl Sepele, Melissa Senne, Logashvari Naidoo, Rachel Kawalazina, Margaret Kasaro, Monica Gandhi, Renee Heffron, Connie Celum
1Makerere University–Johns Hopkins University Research Collaboration, Kampala, Uganda, 2University of Washington, Seattle, WA, USA, 3Setebosh Research Center, Pretoria, South Africa, 4Kenya Medical Research Institute, Nairobi, Kenya, 5Perinatal Research HIV Research Unit of the University of the Witwatersrand, Soweto, South Africa, 6Mabodeng Centre for Research, Bots, South Africa, 7University of the Witwatersrand, Johannesburg, South Africa, 8The Arum Institute, Krugersdorp, South Africa, 9The Arum Institute, Rustenburg, South Africa, 10South African Medical Research Council, Chatsworth, South Africa, 11Kamuzu University of Health Sciences – Johns Hopkins University Research Collaboration, Blantyre, Malawi, 12University of North Carolina in Zambia, Lusaka, Zambia, 13University of California San Francisco, San Francisco, CA, USA, 14University of Alabama at Birmingham, Birmingham, AL, USA
Background: Adolescent girls and young women (AGYW) account for 1 in 5 new HIV infections in sub-Saharan Africa and can greatly benefit from PrEP. While studies among AGYW show high oral PrEP uptake, early discontinuation is common. Objective adherence measures may enhance counselling and promote adherence, but are often costly, require specialized tests and require long turnaround times for spectrometry-based metrics. We evaluated a novel point-of-care urine tenofovir (TFV) assay, using antibody-based technology, to measure adherence and its alignment with self-reported adherence and HIV seroconversion among AGYW.
Methods: From August 2022 to July 2023, we enrolled an open label PrEP cohort of sexually active AGYW aged 16-30 years and interested in PrEP from 20 sites (15 in South Africa and 1 site each in Eswatini, Kenya, Malawi, Uganda, and Zambia). Participants attended study visits 1, 3 and 6 months after enrollment and were offered PrEP and adherence counselling at each visit. PrEP use was assessed via self-report and a qualitative lateral flow urine TFV assay, for which a predetermined threshold of >1500 ng/ml indicates TFV use in the past 4 days. Acceptability of urine TFV testing was assessed at Month 6 via questionnaire.
Results: The INSIGHT cohort enrolled 3087 AGYW. At enrolment, 95.6% of participants initiated PrEP. At months 1, 3, and 6, 95.7%, 94.4%, and 88.8% received PrEP refills and 77.5%, 79.6%, and 64.1% of those with urine tests had TFV detected in the urine assay respectively. The 3 main reasons for PrEP discontinuation were side effects, low risk perception, and peer influence. Self-reported good, very good, or excellent adherence was well aligned with positive results from the urine TFV test (OR=8.5, 95% CI 7.4-9.8). HIV incidence was 1.38/100 person-years (95% CI 0.97-2.08). At Month 6, 58.3% of women reported that a positive urine TFV result motivated them to take PrEP, 23.6% reported that the counsellor helped them identify ways to remember PrEP, and 21% reported that a negative urine test result was not surprising.
Conclusion: Oral PrEP uptake was >95% among a multisite cohort African AGYW with almost 90% refilling PrEP at Month 6 and the majority (64-80%) had evidence of recent use, based on a novel urine TFV assay, which is higher adherence compared to prior studies. Oral PrEP can be an effective PrEP option for African AGYW. Real time drug feedback using the urine TFV assay is acceptable and warrants further study to support PrEP adherence.
169 Phone Calls for PrEP Persistence in Kenyan Women in Postabortal Care: A Cluster Randomized Trial

Renee Heffron1, Lydia Etyan2, Bernard Nyere2, Inviolata Wanyama3, Yasaman Zia4, Torin T. Schaafsma1, Katherine K. Thomas5, Margaret Mwangi6, Lavender A. June7, Felix Mogaka1, Catherine Kiptinness1, Michael Kamiri8, Kenneth Ngure9, Elizabeth Bukusi2, Nelly R. Mugo10
1University of Alabama at Birmingham, Birmingham, AL, USA, 2Kenya Medical Research Institute, Nairobi, Kenya, 3Marie Stopes Kenya, Nairobi, Kenya, 4University of Washington, Seattle, WA, USA, 5Children's Investment Fund Foundation, Nairobi, Kenya, 6Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Background: In Kenya, women of reproductive age face dual epidemics of HIV and unintended pregnancy yet persistence with HIV PrEP is low when initiated in reproductive health settings.

Methods: PrEP delivery was launched in 15 postabortal care (PAC) clinics in Kenya and data were abstracted on women seeking care for 6 months post PAC. Six months after all clinics began to deliver PrEP, we initiated a cluster randomized trial (CRT) and clinics were randomized to conduct either a phone call program (4 calls during the first month, 2 calls in month 2, and 1/month thereafter) or standard of care (SOC) to support PrEP retention and adherence. Data on PrEP refills were abstracted from medical charts. Additionally, women were offered participation in research procedures through which women were tested for tenofovir (TFV) detection using a point-of-care urine assay.

Results: From April 2021 to March 2023, 8362 women sought PAC from participating clinics: median age 24 years (IQR 22-27), 53% married/cohabiting. Of the 15 facilities included, 40% were public and 40% were high volume. The PrEP cascade highlights that 55% received PrEP information, 73% of those had HIV testing, and 36% of those received counseling and initiated PrEP. After the CRT launch, 4112 women sought PAC and 655 (15.9%) initiated PrEP. Overall, 11.8%, 4.9%, and 1.8% of 655 received a PrEP refill at 1, 3, and 6 months after initiation. At month 1, 14/247 (5.7%) women in facilities randomized to SOC and 63/408 (15.4%) of women in the phone call program received a PrEP refill (RR = 2.7, 95% CI 0.90–8.2). At month 1, TFV was detected in 4.9% of the SOC and 11.8% of the phone call arm (RR = 2.4, 95% CI 0.73–8.0), assuming those who did not return were undetectable.

Conclusion: In this adherence cascade study among African women, TFV-DP in DBS was more than one-half lower in pregnant versus non-pregnant women, but PrEM concentrations were similar. Concentrations were lower than levels observed in US cohorts. These benchmarks will help to define women-specific concentration-efficacy relationships and accurate interpretation of HIV prevention trials in African women.

171 HIV Incidence in the INSIGHT Cohort of African Women: Recency Testing and Prospective Follow-Up

Deborah Donnell1, Irene Muku2, Brenda G. Miremb3, Sue Peacec, Harriet Nuwagaba-Birubonwo4, Sinead Delany-Moretlwe5, Katherine Gill6, Pippa MacDonald7, Phillip Kotze8, Alastair van Heerden9, Remco Peters9, Manjeet Jaggernath10, Philipp du Preez11, Renee Heffron12, Connie Kulmu2
1Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 2University of Washington, Seattle, WA, USA, 3Children’s Investment Fund Foundation, Nairobi, Kenya, 4Wits Reproductive Health and HIV Institute, Johannesburg, South Africa, 5Children’s Investment Fund Foundation, Cape Town, South Africa, 6Qhaka Mhlakota Research Clinic, Ladysmith, South Africa, 7Human Sciences Research Council, Pretoria, South Africa, 8Foundation for Professional Development, East London, South Africa, 9University of the Witwatersrand, Johannesburg, South Africa, 10University of Cape Town, Cape Town, South Africa, 11University of Alabama at Birmingham, Birmingham, AL, USA

Background: For efficacy trials of novel agents with an active control, cross-sectional recency testing could estimate HIV incidence in the population screened. There is limited experience with use of recency testing to estimate HIV incidence for this purpose.

Methods: From Aug-Dec 2022, women ages 16-30 were enrolled into the prospective INSIGHT cohort from South Africa (15 sites), eSwatini, Kenya, Malawi, Uganda, and Zambia. Women who screened HIV+ had samples collected for LAg avidity and HIV RNA testing, those with LAg avidity ≤1.5 and viral load (VL) ≥1000 copies/ml were classified as recent infections. Women who were HIV-negative were offered enrollment into an open-label PrEP cohort
for 6 months. At screening, all were asked about their HIV testing history and prior results. Reporting includes only South Africa and Eswatini, where reency testing is complete. Incidence estimates used ABIE v3.

**Results:** In South Africa and Eswatini, 2,682 women were screened (87% of the INSIGHT cohort) and 119 (4.4%) tested HIV-positive, among whom 6 were classified as recent infections (27 had LAg avidity ODn<1.5 and 21 had VL<1000 copies/ml, Figure A), for an estimated HIV incidence rate (IR) of 0.71 (95% CI 0.14-1.28). Among the 2,687 in the HIV-negative cohort followed for 6 months, 97% accepted TDF/FTC PrEP, and there were 17 incident infections, IR=1.23/100 p-y (95% CI 0.77-1.97). 1909 (71%) of this cohort self-reported an HIV test within the past 6 months, including 51 HIV-positive at screening who self-reported a prior HIV negative test (Figure B). Among these 51 possible new infections, 39 had VL obtained; 23/39 (59%) had VL<1000 copies/ml, suggesting ART had been initiated. If the remaining 16/1909 were infections occurring in the prior 6 months, this would be consistent with an IR of at least 1.7/100 p-y, although self-reported test timing and results were unverified.

**Conclusion:** In this first reported use of reency testing during screening of women in Africa, estimated incidence using reency was lower than our observed incidence in prospective follow-up with high PrEP uptake and recent placebo incidence rates of 3-4% in HIV prevention trials. Cross-sectional assessment may have been underestimated due to 1) selection bias if HIV testing is frequent and women with HIV are reluctant to screen, and 2) viral suppression from undisclosed early ART. Understanding sources of bias is critical for refining the reency approach and obtaining accurate contemporary HIV incidence estimates.

![Figure 1.A. Reency testing at Screening and HIV seroconversion during follow-up B. Self-reported recent testing and VL.](image)

**173 Selection of Epigenetically Privileged HIV-1 Proviruses During Treatment With Panobinostat and IFNα**

Marie Armani-Tourret,1 Citapa Adiaya Haranta,1 Isabelle Rosato,2 Leah Carrere, Amy Sbirrella,2 Katrina Shea,2 Theresa Flynn,3 Liliana Velas,4 Alexander Hochroth,5 Frederic D. Bushman,1 Rajesh T. Gandhi,3 Gao Cao, Xu G. Yu,1 Daniel R. Kuritzkes,5 Mathias Lichterfeld,5 Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, Massachusetts General Hospital, Boston, MA, USA, University of Pennsylvania, Philadelphia, PA, USA, Brigham and Women's Hospital, Boston, MA, USA

**Background:** CD4 T cells with latent HIV-1 infection persist despite treatment with currently available antiretroviral agents and represent the main barrier to HIV-1 cure. Pharmacological disruption of viral latency may increase the immunological vulnerability of HIV-1 infected cells, but the efficacy of latency-reversing agents for reducing HIV-1 persistence remains to be proven

**Methods:** We conducted a randomized controlled human clinical trial in which panobinostat (PBT), a potent histone deacetylase inhibitor (HDACi), was evaluated in combination with pegylated IFNα2a (PEG-IFNα2a). ART-treated participants were randomized to receive PBT alone (n=4), the combination of PBT and PEG-IFNα2a (n=9) or PEG-IFNα2a alone (n=4). We quantified CD4 T cell-associated HIV-1 RNA by RT-ddPCR and proviral HIV-1 DNA using the intact proviral DNA assay (IPDA). Cellular immune responses were analyzed by flow cytometry and CD4 T cell gene expression profiling was conducted by RNA-Seq. The integration sites were collected using ISLA or LM-PCR and we conducted a genome-wide assessment of H3K27ac histone marks using CUT&RUN sequencing.

**Results:** The combined treatment with PBT and PEG-IFNα2a increased CD4 T cell-associated HIV-1 RNA (fold increase 1.83, p=0.0029). In parallel, the study medication induced activation of CD4C, pDCs, and cytotoxic NK cells and enhanced the expression of IFNα-stimulated genes. The combined treatment also resulted in a trend for reduced frequencies of intact proviruses, determined by IPDA (p=0.0547). To evaluate effects of the study medication on the proviral landscape, we collected 2,695 integration sites; these studies showed that the combined treatment induced a structural transformation of the HIV-1 reservoir cell pool, characterized by an accumulation of HIV-1 proviruses integrated in ZNF genes (p=0.032), in chromatin regions with reduced H3K27ac marks, and to a lesser extent, in centromeric/satellite DNA regions.

**Conclusion:** Treatment with PBT and PEG-IFNα2a can induce notable changes in the proviral reservoir landscape, with preferential elimination of proviruses in proximity to H3K27ac marks, the molecular target site for PBT. Together, these results provide proof-of-principle that the viral reservoir is vulnerable to “shock and kill” interventions.

**174 Role of Cytoskeleton and Adhesion in a Rare Subset of HIV-Infected Cells That Resist CTL**

Louise Leyre,4 Farah Mustapha,4 Alberto Herrera,4 Paul Zumbo,4 Micheal Galiano,4 Jared Weiler,1 Daron Betel,5 Morgan Huse,1 R. Brad Jones1

Wilen Cornell Medicine, New York, NY, USA, Memorial Sloan Kettering Cancer Center, New York, NY, USA

**Background:** Although latency is a key mechanism of HIV persistence, the reservoir continues to be shaped by cytotoxic T lymphocytes (CTL) pressure under antiretroviral therapy (ART). Growing evidence suggests that this selects for reservoir-harboring cells with CTL-resistant properties, including over expression of BCL-2, PVR and the granzyme B inhibitor SERPINB9. Here, we lay a foundation for identifying novel mechanisms by comprehensively profiling HIV-infected CD4+ T cells that resist CTL in vitro. We uncover and investigate
mechanisms that limit immunological synapse formation – including unique biophysical profiles – as novel modalities of CTL resistance. **Methods:** Central memory CD4+ T cells (Tcm) from each of 4 HIV-viremic donors were divided and infected with either ‘WT’JRCSF or ‘TW10esc’JRCSF with an escape mutation in the Gag-TW10 CTL epitope. These were labeled with CFSE (WT) or CFSE (TW10esc), mixed and cultured with or without TW10-specific CTL clones. Viable HIV-Env+ cells were sorted by flow cytometry into WT ‘Survivors’ (CTFlabeled) and TW10esc ‘Bystanders’ (CFSE) and profiled by RNA-sequencing. Stiffness of Bystander and Survivor cells were measured by optical tweezers, and expression of integrins were quantified by flow cytometry. **Results:** In absence of CTL, WT- and TW10esc-infected Tcm had very similar transcriptional profiles. In striking contrast, the rare (~10%) WT-infected ‘Survivors’ of CTL coculture were divergent from TW10esc-infected ‘Bystanders’ (2,234 DEGs, padj <0.05). Gene ontology highlighted downregulation of genes involved in cytoskeletal regulation in Survivors (NES: -1.59, padj<0.003) and we found by optical tweezers measurements that Survivor cells had a lower stiffness (n=27 mean +/-SEM tether force 82pN [+/-89-74pN]) than Bystanders (n=30 129pN [+/-138-119pN]). As the cytoskeleton plays a pivotal role in modulating integrin activation, cell adhesion genes were also down in survivors (NES: -1.68, padj<0.007). We confirmed using flow cytometry reduced surface expression of ICAM-1 and activation of LFA-1 on Survivors, suggesting impaired adhesion to CTL. **Conclusion:** Killing by CTL requires the formation of immunological synapses which occurs less efficiently with softer target cells. In cancer, lower stiffness and reduced integrin expression are mechanisms of escape. Small molecule and engineered CAR-T therapeutic approaches to target less adherent cells are under development for cancer and should be tested for their abilities to enhance elimination of HIV reservoirs.

**Sex-Specific Innate Immune Selection in Vertical HIV Transmission and cART-Free Aviremia in Males**

Nonomonde Bengu1, Gabriela Z. Cromhout2, Emily Adland1, Katya Gavender3, Nicholas Herbert1, Nicola Cotugno1, Paolo Palm1, Maria C. Puertas1, Thumbi Nhung4, Edmund Capparelli5, Mathias Lichterfeld6, Javier Martinez-Picado7, John Kappers8, Mohendaran Archary9, Philip J. Gouder1

1Queen Nandi Regional Hospital, Empangeni, South Africa, 2University of KwaZulu-Natal, Durban, South Africa, 3University of Oxford, Oxford, United Kingdom, 4Africa Health Research Institute, Mtatutu, South Africa, 5Bambino Gesù Children’s Hospital, Rome, Italy, 6PoxlCoa Institute for AIDS Research, Badalona, Spain, 7University of California San Diego, San Diego, CA, USA, 8Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, 9University of Alabama at Birmingham, Birmingham, AL, USA

**Background:** Following case reports of paediatric post-treatment care, it has been proposed that very early cART without additional interventions might achieve remission in a subset of children. To investigate this possibility, from 2015-2023 we conducted a longitudinal study of >300 mother-child pairs in KwaZulu-Natal, South Africa monitored from birth after in utero HIV transmission. **Methods:** All infants received ART at birth; 92% of infants received transplacental maternal ART pre-birth. ART adherence was assessed via history, pill-counting, pharmacy records and plasma cART concentrations determined by liquid chromatography-tandem mass spectrometry. Chimeric Gag-Protease-NL4-3 viruses were generated following viral RNA isolation and nested RT-PCR amplification of mother and child gag-pro from baseline plasma. Viral type I interferon (IFN-I) sensitivity and replicative capacity were determined using the reporter cell lines U87-snluc/EGFP and CEM-GXR, respectively. **Results:** Despite very early cART initiation, sustained suppression of viremia to 3 yrs was observed in only 32% of children. Aviremia was usually cART-dependent. Unexpectedly, 5 ‘atypical’ males were identified whom aviremia persisted despite complete cART discontinuation for 3m-19m in 4 cases; and 17 m intermittent cART in one case. By contrast, 60% of the cohort was female (p=0.01). Higher in utero transmission rates to female fetuses were only observed in the setting of recent maternal infection (p=0.0055). This was associated with transmission to females of IFN-I resistant (p<0.0001), low replication capacity (‘fitness’) virus (p<0.0001). HIV transmitted to male fetuses was typically IFN-I sensitive/high ‘fitness’. Viruses transmitted to females by mothers who seroconverted in pregnancy were more IFN-I resistant than those not transmitted (p=0.019). Viruses transmitted to males were more IFN-I sensitive than those not transmitted (p=0.02). In sex-discordant twins where only one twin became infected, the female was infected in >90% of cases (p=0.002); the viruses not transmitted to the male twin were more IFN-I resistant than those transmitted to male singletons (p=0.0011). **Conclusion:** These data indicate that early life innate immune sex differences selectively influence vertical HIV transmission and modulate post-treatment control in children living with HIV (figure). The figure, table, or graphic for this abstract has been removed.

176 Sex-Based Differences in HIV-1 Reservoir Profile in Individuals With Long-Term ART Suppression

Toong Seng Tan1, Alexander Hochroth2, Leah Carrere1, Sruthi Kalavacherla3, Gabriela Z. Cromhout4, Paulo Palma3,5, Paolo Palma5, Maria C. Puertas3,6, Thumbi Ndung’u4, Edmund Capparelli5, Mathias Lichterfeld6, Javier Martinez-Picado7, John Kappers8, Mohendaran Archary9, Philip J. Gouder1

1Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, 2amfAR, New York, NY, USA, 3University of California San Francisco, San Francisco, CA, USA, 4Georgetown University, Washington, DC, USA, 5Case Western Reserve University, Cleveland, OH, USA, 6Fredrick National Laboratory for Cancer Research, Frederick, MD, USA

**Background:** Women account for over half of people living with HIV (PLH) but they are largely underrepresented in HIV-1 cure studies. Biological sex impacts host immune responses which may lead to sex-specific selection and evolution of HIV reservoir cells. However, sex-specific differences in HIV reservoir landscapes, including proviral reservoir size, composition, and integration site profile among long-term ART-treated (LT-ART) individuals remain unclear. **Methods:** We included a total of 64 participants (34 males and 30 females, all cisgender), who remained on continuous suppressive ART for a median of 20 (range: 15 – 25) consecutive years with no more than 2 recorded plasma viremia blips (< 100 copies/mL). HIV-1 proviruses and chromosomal integration sites were analyzed using FLIP-seq and MIP-seq, as described in our previous work. **Results:** There were no significant differences in the demographic characteristics between female and male participants in the study. In total, n=4012 HIV genomes were detected in the LT-ART cohort (n=1490 in females and n=2522 in males). Frequencies of total and defective HIV-1 genomes were not different between males and females; however, we found a small trend toward higher frequencies of intact proviruses in females (0.69 vs 0.53 median intact DNA per million PBMC, p = 0.15). Moreover, relative proportions of intact proviruses among total proviruses were higher in females (6.51% vs 3.65%, p < 0.0001). This difference appeared to be at least partially attributable to a higher frequency of clonally-expanded intact proviruses in females compared to males (3.37 vs 0.34 median clonal intact DNA per million PBMC, p = 0.0029). Intriguingly, within a total of 246 integration sites (145 intact, 101 defective) identified, we observed higher proportions of intact proviruses integrated in heterochromatin locations (including centromeric/satellite DNA, ZNF genes) and non-genic DNA in females than males (88% vs 58%, p < 0.0001). **Conclusion:** Taken together, our results suggest a sex-based difference in host immune-driven proviral landscape evolution during long-term suppressive ART. Immune mechanisms responsible for viral reservoir cell selection are unclear at present but may include sex-specific immune responses. The HIV reservoir
in women is associated with features of deeper latency; therefore, women may be primed to achieve a state of HIV control, and the inclusion of women in cure studies should be a priority.

177 AAV-Expressed HIV IgG Biologics Enable Durable ART-Free Viral Control in Infant Macaques

Daniel O’Hagan1, Tracy Ordonez1, Lucas Costa2, Shelpi Pandey3, Siddhartha Shandilya4, Jeremy Smedley5, Diogo M. Magnani6, Deborah Persaud7, Ann Chahroudi8, Matthew R. Gardner9, Michael B. Alpert10, Ann J. Hessell11, Michael Farzan12, Nancy L. Haigwood13, Mauricio A. Martins14

1University of Florida, Gainesville, FL, USA, 2Oregon Health and Sciences University, Portland, OR, USA, 3University of Massachusetts, Worcester, MA, USA, 4The Johns Hopkins University, Baltimore, MD, USA, 5Emory University, Atlanta, GA, USA, 6Yannone, Inc, Jupiter, FL, USA, 7Boston Children’s Hospital, Boston, MA, USA

Background: There were 1.5 million children living with HIV (CLWH) in 2022, only half of whom had access to antiretroviral therapy (ART). Even when ART is available, lifelong daily adherence can be challenging for CLWH, emphasizing the need for alternative strategies to durably suppress HIV replication. Here we evaluated whether a combination of early ART initiation and adenovirus-associated virus (AAV)-vectored delivery of HIV IgG biologics could maintain ART-free viral control in simian-HIV (SHIV)-infected infant rhesus macaques (RMs).

Methods: Ten 4-week-old infant RMs (7 males; 3 females) were orally infected with SHIV-SF162P3 and placed on ART 7 days later, at which time the animals also received intramuscular injections of AAV vectors encoding IgG2 versions of the V3 glycan bnAbs 10-1074 and the immunoadhesin eCD4-Ig. Three control RMs (2 males; 1 female) were similarly infected and placed on ART but did not receive AAV vectors. After 30 weeks, ART was interrupted and the kinetics of virus rebound compared between the two groups.

Results: Peak SHIV plasma viral loads pre-ART ranged from 1.8E+6 to 1.9E+7 vRNA copies/ml. All 10 AAV- treated RMs developed persistent levels of eCD4-Ig in plasma; expression of 10-1074 was more variable due to fluctuating anti-10-1074 antibodies. Following ART interruption (ATI), control RMs became viremic within 2 weeks, whereas 7/10 AAV-treated RMs remained aviremic for over 6 months (P = 0.0005). Low plasma loads of 10-1074 (<6.5 μg/ml) were associated with virus rebound or “blips” in viremia in 3/10 AAV-treated RMs, even when concentrations of eCD4-Ig exceeded 10 μg/ml. One year after ATI, two aivircemic experimental RMs were treated with a neonatal Fc receptor (FcRn)-blocking antibody to probe the mechanism of virus suppression. FcRn blockade markedly reduced plasma levels of total IgG, including eCD4-Ig and 10-1074, resulting in breakthrough viremia 2 weeks later, thus demonstrating the direct role of AAV-expressed HIV IgG biologics in ART-free viral control.

Conclusion: A one-time dose of AAV vectors given to 5-week-old infant RMs starting ART early after SHIV infection was safe and resulted in levels of eCD4-Ig and 10-1074 that prevented virus rebound and maintained virus control for up to 1 year post ATI. Maintenance of virus control required continuous expression of both eCD4-Ig and 10-1074 in plasma at ≥6.5 μg/ml. In sum, AAV-vectored delivery of HIV biologics holds promise for achieving sustained virologic remission in CLWH in a practical and scalable manner.

178 Broadly Neutralizing Antibody-Secretory T Cells and CAR-Ts Potently Suppress In Vivo HIV Infection

Hang Su1, Jenny Zheng2, Scott Garforth3, Kim Anthony-Gonda4, Rimas J. Orentas5, Siddhartha Shandilya6, Francesca Marzullo7, Luis J. Montaner8, Suvadip Mallick1, Matteo Forini1, Karam Mounzer9, Pablo Tebas10, Luís J. Montaner11, Mathias Lichterfeld12, Katherine J. Barrett13

1University of Pennsylvania, Philadelphia, PA, USA, 2Harvard Medical School, Boston, MA, USA, 3Wistar Institute, Philadelphia, PA, USA, 4Philadelphia FIGHT, Philadelphia, PA, USA

Background: Broadly neutralizing antibodies (bNAb) are being tested in clinical trials of treatment and cure in people with HIV (PWH) who have existing autologous neutralizing antibody (anAb) responses. To determine the relative protective pressure of bnAbs and anAbs in the BEAT2 study of 3BNC117, 10-1074, and IFNa2b, we performed a sieve analysis comparing the potency of administered bnAbs and host anAbs against reservoir and rebound Env.

Methods: In 8 participants of BEAT2 (NCT035588715), we sequenced proviruses from PBMCs collected at study enrollment via FLIPSeq or MiPSeq (n = 314) to identify intact HIV-1 genomes in single and clonally-expanded populations. In 12 participants, plasma rebound envs (n = 223) were sequenced by SGS at first detectable rebound viremia. Reservoir and rebound Envs were cloned and tested for neutralization sensitivity via TZM.bl assay to bnAbs and longitudinal plasma IgG (collected at a median of 8 time points over 12-24 study months). Statistical comparisons were with Wilcoxon matched-pairs test.

Results: In all 8 participants, rebound envs aligned within reservoir phylogenies, but were not identical to any sampled reservoir viruses. Comparing rebound Envs (1 per participant) and reservoir Envs (2-6 per participant), rebound Envs were significantly more resistant to 10-1074 (IC50, 6.44 μg/ml vs. 0.71 μg/ml; p = 0.02), and trended towards greater resistance to 3BNC117 (ns). When tested against baseline plasma IgG (prior to bnAb dosing), rebound Envs were generally more resistant than reservoir Envs, with markedly greater resistance (30-fold difference in IC50) in the 2 participants with most delayed rebound (>12 weeks post-bnAb dosing). Potency of baseline IgG against both rebound and reservoir Envs rose significantly during bnAb dosing (mean >3-fold change in IC50 for both; p = 0.001). Post-ART restart, after bnAbs had waned and anAbs had responded to recent rebound viremia, plasma IgG potency increased vs. rebound Envs (mean 2.5-fold change in IC50; p = 0.001), but not reservoir Envs (ns).

Conclusion: In the BEAT2 study of 2 bnAbs and IFNa2b, the greater potency of the administered bnAbs against reservoir vs. rebound Envs indicates bnAb selective pressure. In 2 participants with delayed rebound, baseline anAbs also exerted selective pressure. After rebound and ART restart, anAbs evolved to selectively target rebound Envs. Together, results suggest that anAbs contributed to virus suppression in a subset (25%) of studied bnAb trial participants. Approaches to boost anAbs may increase this proportion.

199 Autologous Neutralizing Antibodies Contribute to Virus Control in a Subset of PWH Treated With bnAbs

Francesco E. Marino1, Maxime Belfroid2, Ryan Krause1, Marie Armanzi-Tourret3, Suvadip Mallick1, Emmanuel Papasavvas4, Matthew Faire4, Karam Mounzer5, Pablo Tebas5, Luis J. Montaner5, Mathias Lichterfeld6, Katherine J. Barret6

1University of Pennsylvania, Philadelphia, PA, USA, 2Harvard Medical School, Boston, MA, USA, 3Wistar Institute, Philadelphia, PA, USA, 4Philadelphia FIGHT, Philadelphia, PA, USA

Background: BnAbs are being tested in clinical trials of treatment and cure in people with HIV (PWH) who have existing autologous neutralizing antibody (anAb) responses. To determine the relative protective pressure of bnAbs and anAbs in the BEAT2 study of 3BNC117, 10-1074, and IFNa2b, we performed a sieve analysis comparing the potency of administered bnAbs and host anAbs against reservoir and rebound Envs.

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Results: In all 8 participants, rebound envs aligned within reservoir phylogenies, but were not identical to any sampled reservoir viruses. Comparing rebound Envs (1 per participant) and reservoir Envs (2-6 per participant), rebound Envs were significantly more resistant to 10-1074 (IC50, 6.44 μg/ml vs. 0.71 μg/ml; p = 0.02), and trended towards greater resistance to 3BNC117 (ns). When tested against baseline plasma IgG (prior to bnAb dosing), rebound Envs were generally more resistant than reservoir Envs, with markedly greater resistance (30-fold difference in IC50) in the 2 participants with most delayed rebound (>12 weeks post-bnAb dosing). Potency of baseline IgG against both rebound and reservoir Envs rose significantly during bnAb dosing (mean >3-fold change in IC50 for both; p = 0.001). Post-ART restart, after bnAbs had waned and anAbs had responded to recent rebound viremia, plasma IgG potency increased vs. rebound Envs (mean 2.5-fold change in IC50; p = 0.001), but not reservoir Envs (ns).

Conclusion: In the BEAT2 study of 2 bnAbs and IFNa2b, the greater potency of the administered bnAbs against reservoir vs. rebound Envs indicates bnAb selective pressure. In 2 participants with delayed rebound, baseline anAbs also exerted selective pressure. After rebound and ART restart, anAbs evolved to selectively target rebound Envs. Together, results suggest that anAbs contributed to virus suppression in a subset (25%) of studied bnAb trial participants. Approaches to boost anAbs may increase this proportion.
180 A T Cell-Targeting mRNA SIV Vaccine Extends Time to Rebound and Enhances Post-ART Viral Control

Robert W. Omame, Benjamin Varco-Merth, Omo Fadeyi, William Goodwin, Alejandro Marenco, Derek Dull, Jeremy Smalley, Michael Axthelm, Brandon Keel, Jeffrey Litsion, Janina Gergen, Susanne Rauch, Benjamin Petch, Louis Picker, Alam A. Okoye

Emory University, Atlanta, GA, USA, University of Texas at Dallas, Dallas, TX, USA, Charité University Medicine Berlin, Berlin, Germany

Background: Natural CD8+ T cell responses are ineffective at intercepting rebounding reservoirs after antiretroviral therapy (ART) release until after systemic viral replication and spread. Here, we evaluated whether a nucleoside unmodified mRNA vaccine (RNActive®) expressing full length SIV Gag and formulated in lipid nanoparticles (LNP) can be used to enhance Gag-specific CD8+ T cells in SIVmac239-infected rhesus macaques (RM) on ART. We hypothesized that vaccination during ART followed by a boost just prior to ART interruption (ATI) will enhance the immune intercept of reactivating SIV infections and facilitate post-ART viral control.

Methods: 16 RMs were infected with 5000IU of SIVmac239M and started on ART 4 days post-infection. Following viral suppression, RM were divided into 2 balanced groups that received 5 intramuscular injections at 100μg each of the SIV-Gag (n=8) or control mRNA/LNP vectors (n=8) at 59-, 62-, 65-, 79- and 106-weeks post-infection (wpi). SIV-specific T cell responses were assessed by intracellular cytokine stimulation assay. Two weeks after the last immunization, at 108wpi, ART was stopped to assess the impact of vaccine-induced immune responses on post-ART viral replication using RT-PCR.

Results: SIV-Gag mRNA/LNP increased Gag-specific CD8+ T cells in multiple tissues, particularly the bronchoalveolar lavage (BAL), which saw responses peak to 25% (mean ± 4.4% SEM) of CD8+ T cells. Indeed, overall frequencies of Gag-specific CD8+ T cells in BAL were higher in SIV-Gag mRNA/LNP vaccinated RM relative to controls after 47 weeks (AUC, p<0.0002). A boost SIV-Gag mRNA/LNP at 16 days prior to ATI significantly increased frequencies of Gag-specific CD8+ T cells in blood (p=0.0002), BAL (p=0.0002), lymph node (p=0.0003), bone marrow (p=0.0002), spleen (p=0.0002), rectum (p=0.02) and liver (p=0.0002) relative to controls. Upon ART cessation, time to viral rebound was delayed in RM that received the SIV-Gag mRNA/LNP (median 20 vs. 11.5 days, p=0.0005) (Figure 1A). Additionally, SIV-Gag mRNA/LNP RM had lower post-ART peak viremia (p=0.0002) and lower viral burden up to 5 weeks after rebound (AUC, p=0.0002) (Figure 1B).

Conclusion: Collectively, these data suggest that a T cell-targeted vaccination strategy that systemically increases the frequencies of SIV-specific CD8+ T cells immediately prior to ATI can restrict early viral spread and facilitate enhanced post-ART viral control.

181 IL-15/IL-15Ra Cytokine Therapy Enhances Control of Viral Rebound in SIV-Infected Macaques


Emory University, Atlanta, GA, USA, University of Louisiana at Lafayette, Lafayette, LA, USA

Background: Immunotherapeutic cytokines can enhance immune responses against chronic infections. Cytokines such as IL-15 and IL-12 expand CD8 T and NK cells and increase their cytotoxicity. Importantly IL-15 can enhance follicular homing of CD8 T cells, and IL-15 + IL-12 enhances follicular homing of NK cells during chronic SIV infection. Here we tested the therapeutic effects of IL-15 and IL-12 when administered alone or in combination during chronic SIV infection in rhesus macaques (RMs).

Methods: Twenty-two RMs infected with SIVmac251 were initiated on antiretroviral therapy (ART) at 8 weeks post-SIV infection for 9-months. Three groups of animals received cytokine treatments - IL-15/IL-15Ra (n=6), IL-12 (n=5), IL-15/IL-15Ra+IL-12 (n=6) – in two phases (5 doses once a week). The first phase was administered at 6 weeks post SIV (2 weeks prior to ART) and the second phase was during ART at 12 weeks pre- ATI. ART alone (n=5) group served as the control. Animals were monitored longitudinally for immunological and virological parameters.

Results: IL-15/IL-15Ra and IL-15/IL-15Ra+IL-12 therapies induced significant expansion of functional SIV-specific CD8 T cells with proliferative capacity (Ki-67) and follicular homing (CXCR5) and CD16+ NK cells in blood and LN. Importantly, IL-15/IL-15Ra therapy resulted in the significant expansion of degranulating CD107a+CD8 T cells pre-ATI (p<0.03). In addition, the blood transcriptomic profile confirmed the induction of cytolytic molecules (granzyme-B, perforin), Jak/Stat signaling pathway in IL-15/IL-15a group, while genes associated with cell cycle arrest, DNA damage (Mdm2, Ddit4) were significantly reduced. Post-ATI, virus rebounded in all animals but IL-15/IL-15Ra treated animals showed nearly 3-log lower viremia compared to ART only animals (p=0.004) with 83% of animals below 500 copies/ml at 20 weeks post ATI. This level of viral control was not observed in IL-12 or dual cytokine treated group. SIV-specific CXCR5 + CD8 T cells in blood (p=0.04) and LN (p=0.02) and SIV-specific CD28+ cells in blood (p=0.002 and LN (p=0.03) were associated with control of viremia post ATI.

Conclusion: The IL-15/IL-15Ra therapy at the initiation of ART and during ART markedly enhance the magnitude and function of SIV-specific CXCR5+CD8 T cells and CD16+ NK cells and contribute to profound control of viremia post ATI. These studies define IL-15/IL-15Ra as a potentially effective immune therapy for HIV cure strategy.

182 High Rates of Viral Suppression in Pregnancy Drop Postpartum in South African Women on TLD

Elaine J. Abrams, Jennifer Iao, Elton Mukonda, Hlengwe Madlala, Phindi Zwane, Jack Hu, Allison Zerbe, Justine Legbedze, Landon Myer

University of Cape Town, Cape Town, South Africa

Background: The global transition to 1st-line antiretroviral treatment (ART) with tenofovir+lamivudine+dolutegravir (TLD) has shown high rates of viral suppression (VS) in adults and children but little is known about pregnant and postpartum women.

Methods: Pregnant women with HIV (PWH) already on TLD (Continuers, PWHc) or starting TLD <14 days prior to enrollment (Initiators, PWHi) were enrolled
in ORCHID, an observational study of metabolic health, in Cape Town, South Africa, Sept 2021-Sept 2023. PWH were enrolled <18 weeks (wks) gestational age (GA); ART was managed by routine clinical services; viral load (VL) samples were collected at enrollment, trimester 2 (T2, 24-28 wks), T3 (32-34 wks) and T6-12 wks postpartum (PP). Analyses described VS (<50 cps/mL), the incidence of major (>1000 cps/mL) and minor (50-1000 cps/mL) viremic episodes (VE) and associated factors among PWH using Poisson models.

**Results:** Among 600 PWH, 450/545 PWH, median (IQ) GA age was 30.0 yrs (10-47), GA was 13 wks (10-16), and duration on TLD was 218 days (15-554) at enrollment (366 [149-716] and 10-7) for PWH and PWH respectively. Median VL at enrollment was 19 cps/mL (range 19-980,148); 475 (79%) PWH had VL<50 cps/mL (89%PWH, 49%PWH), 76 (13%) had 50-1000 cps/mL (PWH 7%, PWH 29%) and 49 (8.2%) had >1000 cps/mL (PWH 3.8%, PWH 2%) [Fig1].

Overall, 3142 woman-months of observation were accrued: 567 (95%) PWH had ≥1 VL <50 cps/mL; of these women 45 (8%) had ≥1 minor VE (8% vs 7%, p=0.8) and 39 (7%) had ≥1 major VE (4%/ vs 15%, p<0.001). The proportion of VL measures with VS increased from enrollment (79%), was high at T2 (91%), T3 (90%), and 6wks PP (91%) but decreased thereafter. By 24wks PP, 21% of 127 VL measures were >1000cps/mL, (12%/ vs 33%/p=0.007). In multivariable analyses the incidence of VE increased above enrollment (79%), was high at T2 (91%), T3 (90%), and 6wks PP (91%) but decreased thereafter. By 24wks PP, 21% of 127

**Methods:** Elevated BP and hypertensive disorders during pregnancy and postpartum. We performed a post-hoc analysis of blood pressure (BP) data collected in IMPAACT 2010 to characterize by-arm incidence of elevated BP and hypertensive disorders during pregnancy and postpartum. We found high rates of VS in pregnant women, but postpartum viremia remains a pressing concern, particularly for younger women and those initiating ART during pregnancy.

**Results:** 626 participants were included: 211 in DTG+F/TFA, 208 in DTG+F/TDF, and 207 in EVF/F/TDF (11 were excluded for HTN at entry). Baseline medians were: age 26.4 yrs, GA 21.9 wks, HIV RNA 938 cps/mL, CD4 cell count 472 cells/μL, BMI 24.6 kg/m². Incident elevated BP or HTN (mild+) was high overall (55%) and more common with DTG+F/TAF (59%) and DTG+F/TDF (56%) relative to EVF/F/TDF (51%). Moderate and severe HTN occurred in 1.6% of women (Table). 12 women had pre-eclampsia and 1 had eclampsia, with no apparent pattern by arm. While the estimated difference between DTG arms was small, there was a trend toward an increased hazard of incident elevated BP or gestational or non-gestational HTN for DTG+F/TAF vs EVF/F/TDF (HR 1.26, 95%CI 0.98, 1.64) and DTG+F/TDF vs EVF/F/TDF (HR 1.18, 95%CI 0.9,1.53); results adjusted for time-varying weight were similar.

**Conclusion:** Our data are consistent with findings that DTG-based ART may be associated with incident HTN, largely accounted for numerically more women with BP ≥ 130-139/80-89mmHg in this cohort of young, pregnant and postpartum women. Our findings should be confirmed with additional studies. Pending further data, efforts should focus on early identification and management of hypertensive disorders in pregnant and postpartum women on DTG.

**Figure 1:** Distribution of viral load end points during pregnancy and postpartum among women entering pregnancy or TLD (continues; see also Table 1 for administration).
Results: Six children underwent ATI at median age 5.5 years. Three of 6 achieved study-defined remission, one through 80 weeks of ATI, when viral rebound (299,538 cp/mL) occurred. The other two who achieved remission remain on ATI (>48 and >60 weeks). A fourth child remains on ATI (>44 weeks). Two children had viral rebound 3 and 8 weeks after ATI (Table). Earliest available HIV-1 RNA and DNA values ranged from 96 to >5 million cp/mL and from not detected to 130 cp/10^6 PBMCs. The child with 80 weeks of remission had no ARVs detected in plasma during ATI (tests pending for others). Two of 3 children with rebound (5 and 80 weeks) experienced acute retroviral syndrome (ARS); no other clinical or immunologic events of concern were identified during or following ATI. The children with rebound at 3 weeks (67,606 cp/mL) and 8 weeks (1801 cp/mL) had HIV-1 RNA <LOD 8 weeks and 20 weeks after resuming ART. The child with rebound at 80 weeks had HIV-1 RNA 724 cp/mL 2 weeks after resuming ART.

Conclusion: ART-free remission for >48 weeks was achieved with very early treatment of in utero HIV-1. Very early treatment with durable virologic suppression may enable sustained remission in children; however, the occurrence of 80 weeks of ART is not rare in African children with HIV/AIDS.
Emerging Dolutegravir Resistance Among Children Being Investigated for Treatment Failure in Malawi

George Bello1, Sherri Pails2, Barbara Bighignoli3, Alinune Kabaghe4, Jonathan Mungudza5, Salee Panji6, Elliot Baizer7, Elizabeth Kamphira8, Dambani Kambira9, Bilial W. Matola10, Stephanie Hackett11, Duping Zheng12, Bianca Alvarez13, Nelliie Wadona-Kabondo14, for the HIV Drug Resistance Surveillance Team

1International Training and Education Center for Health, Pretoria-Villa, Haiti; 2US Centers for Disease Control and Prevention, Winthrop, Maryland; 3Centers for Disease Control and Prevention, Atlanta, GA, USA; 4Centers for Disease Control and Prevention, Lilongwe, Malawi; 5Government of Malawi Ministry of Health, Lilongwe, Malawi

**Background:** Malawi switched from protease inhibitor- (PI) and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based paediatric first- and second-line antiretroviral therapy (ART) regimens to dolatregravir-based regimens (DBR) in 2020. By 2022, over 98% of children living with HIV (CLHV) were on DBR, requiring monitoring of dolatregravir (DTG) resistance. We evaluated the prevalence and patterns of drug resistance (DR) to DTG in children in Malawi.

**Methods:** We conducted a cross-sectional survey in 19 clinics randomly selected from the 25 highest volume ART clinics in Malawi from November 2022 to March 2023. We included CLHV aged <15 years, on a DR <9 months, returning to the clinic after a previous high viral load (VL) >1000 copies/ml and having completed at least 1 session of intensive adherence counselling (IAC) per national guidelines. A plasma sample was obtained for VL re-testing. Samples with VL ≥ 1000 copies/ml were genotyped for DR using HIV-1 Genotyping kit with Integrase (Thermofisher) and interpreted using Stanford University HIVDR Database Algorithm (version 9.4). We present weighted estimates of DR (level 3-5) with 95% confidence limits accounting for correlation within clinics using SAS.

**Results:** Of the 297 CLHV re-tested for VL, 43.1% (128/297) remained unsuppressed. Of the 128 CLHV that remained unsuppressed, 97.7% (125/128) were successfully genotyped for DR mutations (DRMs). Of those successfully genotyped, median age was 10 years old (IQR 5-13); 58% were male, median time since ART initiation was 5.4 years (IQR 2.5-10.0); median time on DTG was 1.5 years (IQR 1.2-2.3); and 89% were ART-experienced at DTG initiation. The weighted prevalence of high-level DTG resistance among children with virological failure was 15.5% (95% CI: 6.7-24.3). The most common major DTG DRMs were R263K (10), E138K/A (5), S476G (4), and G119R (4). Resistance to any nucleoside reverse transcriptase was 41.1% (95%CI: 27.6-54.6); to any NNRTI was 65.0% (95%CI: 53.8-76.2); and any PI was 5.2% (95%CI: 0.0-12.2).

**Conclusion:** Among Malawian CLHV with confirmed virological failure on DTG, DTG DRM prevalence was 15.5%, twice as high as the 8.5% found in a parallel study among Malawian adults. Prevalence of DR to PI was rare. These collective results raise concern about effective future treatment of CLHV, as there are no convenient alternative 2nd or 3rd line ART options currently available for this population.

Long-Acting Cabotegravir Plus Rilpivirine In Adolescents With HIV: Week 4 IMPAACT 2017 (MOCHA) Study

Aditya Gaur1, Edmund Capparell2, Kristin Baltusris3, Mark Minzkie4, Conn M. Harrington1, Cindy McGic5, Herta Crauwell6, Ellen Townley7, Jack Moye8, Sarah Buisson9, Ayv Violar10, Pradthana Dunchanam11, Chelsea Kroette12, Carolyn Bolton13, for the IMPAACT 2017 Team

1St Jude Children’s Research Hospital, Memphis, TN, USA; 2University of California San Diego, La Jolla, CA, USA; 3Harvard TH Chan School of Public Health, Boston, MA, USA; 4Johns Hopkins University School of Medicine, Baltimore, MD, USA; 5VA Healthcare, Research Triangle Park, NC, USA; 6VA Healthcare, Madison, Spain; 7Jaarsen Research & Development, LLC, Pennington, NJ, USA; 8National Institute of Allergy and Infectious Diseases, Washington, DC, USA; 9National Institute of Child Health and Human Development, Bethesda, MD, USA; 10FHI 360, Durham, NC, USA; 11Chulalongkorn University, Bangkok, Thailand; 12Chiang Mai University, Chiang Mai, Thailand; 13Frontier Science & Technology Research Foundation, Inc, Amherst, NY, USA; 14Centre for Infectious Disease Research in Zambwa, Lusaka, Zambia

**Background:** Long-acting (LA), intramuscular (IM) cabotegravir (CAB) + rilpivirine (RPV) constitutes the first LA combination antiretroviral treatment (ART) regimen for people with HIV-1. The goal of the ongoing IMPAACT 2017 study (MOCHA [More Options for Children and Adolescents]; NCT03497676) is to evaluate the safety, tolerability, and pharmacokinetics (PK) of this LA combination in virologically suppressed (HIV-1 RNA < 50 copies/ml) adolescents. Here we present PK and safety data through the primary Week 24 timepoint and available safety data beyond Week 24.

**Methods:** In this Phase I/II, open-label, noncomparative trial, virologically suppressed adolescents (12 to <18 years of age; ≥ 35 kg) with HIV-1 switched from their pre-study ART to 4 weeks of daily oral CAB + RPV followed by 600 mg CAB LA and 900 mg RPV LA IM (3 mL each) in the contralateral gluteal muscles per the every 2-month dosing regimen. The 1st and 2nd injections were 4 weeks apart, with subsequent injections every 8 weeks.

**Results:** 144 participants were enrolled at 18 sites in 5 countries. Median (min, max) age was 15 years (12, 17), body mass index 19.5 kg/m² (16, 34), weight 48 kg (35, 103), 49% male and 74% Black or African American. Most participants received ≥1 injection 142/144 (100%) and completed the Week 24 visit (141/144); mean (standard deviation) study duration was 56 weeks (13). No deaths or adverse events (AEs) leading to study drug discontinuation occurred; no serious AEs attributable to study product occurred. Through Week 24, 16/144 (11%) had a ≥ Grade 3 AE, most common being increases in blood creatine phosphokinase (n=6) and systolic blood pressure (n=3); none of these non-injection site reaction (ISR) AEs were considered study drug related. For all safety data, 49/142 (35%) participants reported an ISR, most (86%) ISRs resolved within 7 days and were Grade 1 (91%). Two of 144 (1%) participants experienced a drug-related ≥ Grade 3 AE (injection site pain and abscesses [n=1]; injection site abscesses [n=1]). The outcome of the single unintended pregnancy in a study participant was a healthy live birth. There was no virologic failure through Week 24. The median (5%, 95%) Week 24 predose concentrations for CAB (2.34 μg/ml [1.11, 4.15]) and RPV (49.5 ng/ml [25.9, 78.1]) were similar to those in adults (Figure). One participant had low CAB concentration at Week 24 (0.03 μg/ml).

**Conclusion:** IMPAACT 2017 (MOCHA) data support using CAB-LA plus RPV-LA every 2 months in virologically suppressed adolescents.

**SEARCH: Youth Intervention Impact on Symptoms of Depression in East African Young Adult Women Living With HIV**

Florence Mwanga1, James Peng2, Laura B. Baizer3, James Ayien4, Janice Litunya5, Jason Johnson-Peretz6, Douglas Black7, Janet Nakigudde, Elizabeth Bukusi, Moses R. Kamya8, Theodore Ruel9, Diane V. Hawli10, Carol S. Camlin11

1Infectious Diseases Research Collaboration, Kampala, Uganda; 2University of Washington, Seattle, WA, USA; 3University of California Berkeley, Berkeley, CA, USA; 4Kenya Medical Research Institute, Kilifi, Kenya; 5University of California Los Angeles, Los Angeles, CA, USA; 6University of California San Francisco, San Francisco, CA, USA; 7Makere University College of Health Sciences, Kampala, Uganda

**Background:** Depression is common among youth with HIV and is associated with adverse outcomes. The SEARCH Youth intervention included a life-stage based assessment of psychosocial issues in youth with HIV. We sought to determine if the intervention affected the prevalence of depressive symptoms.

**Methods:** SEARCH Youth was a cluster-randomized trial of youth aged 15-24 years in 28 clinics of rural Kenya and Uganda that demonstrated increased viral suppression at 2 years. Intervention clinics utilized a life-stage discussion tool with adverse outcomes. The SEARCH Youth intervention included a life-stage assessment of psychosocial issues in youth with HIV. We sought to determine if the intervention affected the prevalence of depressive symptoms.

**Results:** Of the 1,811 eligible, 662 intervention and 572 control participants were assessed after a median 3.8 years of follow up. Median age was 21 years, and 80% were female with baseline characteristics balanced by arm. Overall, 53% of the intervention arm compared to 73% in the control had any depressive symptoms, representing a 28% risk reduction [relative risk:0.72 (95% CI: 0.59-0.89)]. There was a trend to risk reduction for at least mild (0.45; 0.13-1.57) or moderate-severe (0.48; 0.10-2.29) depression in the intervention arm. Across subgroups, the intervention conferred risk reduction for any depressive symptoms with the greatest effects among participants in Kenya (0.54; 0.38-
0.79) and those re-engaging in care (0.67;0.55-0.82). Predictors (odds ratio; 95% CI) of at least mild depression included feeling sexual pressure (10.6;3.8-29.4), feeling physically threatened (6.3;3.3-12.5), and recent life events (4.2;2.6-6.7), including sickness (4.1;2.4-7.0) or family death (7.5;4.0-14.1).

Conclusion: The SEARCH Youth intervention reduced the prevalence of depressive symptoms particularly for those re-engaging in care. Recent major life events and the perception of sexual or physical threat were key drivers of depression in this population. We postulate that life-stage based discussions helped providers and patients identify and navigate challenging issues, building resilience against both depression and lapses in adherence and care.

190 Role of Community-Level Factors in Declines in HIV Incidence and Prevalence Among Rakai Adolescents

Stephanie A. Griolo1, Julia Thompson2, Ivy S. Chen1, Fred Nalugoda4, Tom Latulipe5, Ying Wei5, Esther Spindler5, Susie Hoffman6, Philip Kireke6, David Serwadda4, Mary Kate Grabowski1, Maria J. Wawer1, Fred M. Ssewamala2, Larry W. Chang3, John S. Santelli5 (Columbia University Medical Center, New York, NY, USA; Rakai Health Sciences Program, Kalisizo, Uganda; Johns Hopkins University, Baltimore, MD, USA; Washington University in St Louis, St Louis, MO, USA)

Background: HIV acquisition among adolescents (15-19 years) and young adults (20-24 years) is influenced by individual factors, community factors, and public policies and programs. We explored the association of HIV incidence and prevalence with these factors over time among adolescents and young adults (AYA) in Rakai, Uganda.

Methods: We examined trends among AYA (n= 35,938 person rounds) from nine survey rounds (2005-2020) of the Rakai Community Cohort Study (RCCS), a population-based open cohort of individuals living in 30 continuously followed communities in southcentral Uganda. We evaluated the impact of community viremia (CV, a measure of community-level ART use and HIV prevalence) on HIV incidence and prevalence among AYA. Logistic GEE, Poisson GLM and univariate models were run for HIV prevalence, HIV incidence, and predictors of interest, respectively.

Results: HIV incidence and prevalence declined after round 14 (2010-2011) by 66% among AYA men and after round 17 (2015-16) by 60% among AYA women.

Between survey round 11 and round 19, the proportions reporting sexually experienced declined from 58% to 38% in adolescent men and from 65% to 35% among adolescent women. The prevalence of male medical circumcision (MMC) among AYA men increased from 20% in round 11 to 79% in round 19. At the community-level, we found substantial increases in ART use among PLHIV among communities that were already using ART by round 11 (15% in round 11 and 86% in round 19) and corresponding declines in CV. In multivariable analyses, a combination of individual and community-level factors were found to predict HIV incidence and prevalence among AYA, notably MMC among young men and CV among young women.

Conclusion: Declines in HIV incidence and prevalence occurred first among AYA men and later among AYA women. These coincided with declines in sexual experience and with public policies to increase access to MMC and ART. Combination HIV prevention with AYA needs to address risk factors at multiple levels. Individual risk behaviors continue to play a role in HIV incident and prevalence infection. Thus, it remains important to have conversations with AYA about their individual behaviors. However, community level factors are playing an important role and therefore these conversations should occur within the context of larger social forces of transmission risk.

191 Investigation of HIV Transmission Associated With Receipt of Vampire Facials: New Mexico, 2018-2023

Anna M. Behar1, Mika N. Gehre2, Liana Atallah3, Tegan Clarke4, Ana-Alicia Leonso1, Francisco Jojola4, Haoqiang Zheng5, Hongwei Jia5, Scott Peter C. Grytdal1, Miranda Durham1, N. Mariam Salas2, Maria J. Wawer1, Fred M. Ssewamala2, Larry W. Chang3, John S. Santelli5 (Columbia University Medical Center, New York, NY, USA; New Mexico Department of Health, Santa Fe, NM, USA; University of New Mexico, Albuquerque, NM, USA; Albuquerque Public Schools, Albuquerque, NM, USA; Indian Health Services, Carroll, NM, USA)

Background: HIV transmission through cosmetic injection services via contaminated blood has not been previously documented in the United States. In summer 2018, the New Mexico Department of Health (NMDOH) was notified of a diagnosis of HIV infection in a female with no known HIV risk factors who reported exposure to needles from cosmetic platelet-rich plasma micro-needling (vampire facial) received at a spa in spring 2018.

Methods: This report led NMDOH and CDC to investigate possible transmission of HIV through cosmetic injection services. The period of interest for active case finding was from spring 2018, when the initial case received a vampire facial, to fall 2018 when the spa closed, and on-site inspection of the spa was conducted. Names and phone numbers were compiled and cross-referenced from spa client consent forms, handwritten appointment records, and cell phone contacts to form a list of potentially affected clients who were directly contacted to encourage testing for bloodborne pathogens. From 2018-2023, suspected cases were reported to NMDOH from clinical providers throughout the state, and blood specimens were submitted to CDC for nucleotide sequence analysis (NSA) to determine cluster association.

Results: Active case finding identified one client with a previous diagnosis of HIV in 2012, 20 clients who received vampire facials, and 59 clients who received other injection services (e.g., Botox) during spring-fall 2018. Among the 198 former spa clients and their sexual partners tested during 2018-2023, no new HIV, Hepatitis B, or Hepatitis C infections were identified. The on-site inspection revealed several unsafe infection control practices including storage of unlabeled tubes of blood on the kitchen counter. Five suspect cases, four former spa clients plus one sexual partner of a spa client, were reported to NMDOH all of whom had HIV diagnosed during 2018–2023 and no known HIV risk factors. NSA revealed highly similar HIV strains among all cases indicating vampire facials as the likely transmission route of HIV for three cases in this cluster. The other two cases, who had previous HIV infections, were likely attributed to sexual contact. Sequences from the former client living with HIV did not cluster with any sequences from cases.

Conclusion: This investigation underscores the importance of assessing novel sources of HIV transmission among persons with no known HIV risk factors, and adequate infection control practices at facilities offering cosmetic injection services.

192 Trends in Black-White Disparities in HIV Diagnosis: 2017-2021, United States

André Dailey1, Janetta Gant Sunner2, Anna Satchter Johnson1, Juliet A. Morales3, Sue Reynolds4 (Centers for Disease Control and Prevention, Atlanta, GA, USA)

Background: The largest disparities in HIV diagnoses in the United States are between Black and White persons. Federal initiatives for HIV prevention have evolved over the years, with the 2025 National HIV/AIDS Strategy including a focus on health equity. We examined trends in Black-White HIV diagnosis disparities to evaluate progress towards achieving equity in HIV diagnosis in the United States.

Methods: Data from CDC’s National HIV Surveillance System were used to assess temporal trends in absolute and relative disparities in HIV diagnosis between Black and White persons during 2017–2021. Predicted values based on four years (2017–2019 and 2021) of data were used. Data for the year 2020 were excluded due to the impact of COVID-19 on HIV diagnoses. Estimated annual percentage change (EAPC) and 95% confidence intervals (CIs) were calculated to assess trends by selected characteristics.

Results: Between 2017 to 2021, absolute disparities in Black-White HIV diagnosis decreased among males from 65.7 per 100,000 population to 57.6 per 100,000 population (absolute: EAPC = -3.2 [CI: -3.6, -2.7]) and from 22.5 per 100,000 population to 17.7 per 100,000 population (absolute: EAPC = -5.8 [CI: -6.5, -5.0]) among females. Relative disparities decreased from 15.1 per 100,000 population to 10.8 per 100,000 population among females (EAPC = -7.2 [CI: -8.4, -5.9]) and remained the same for males. Among male subpopulations, disparities increased among those with HIV attributable to male-to-male sexual contact (Absolute: EAPC = 1.2 [CI: 0.8, 1.5]; Relative: EAPC = 1.5 [CI: 1.0, 1.9]).

Sonia Singh, Xiaohong Hu, Kristen L. Hess, Kashif Iqbal

Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Estimates of lifetime risk are used to compare the burden of disease across populations. This method may be a useful tool for clinicians, outreach workers and policy makers when describing the burden of HIV since it can be more easily understood by the general population. We estimated lifetime risk of a HIV diagnosis among MSM by race/ethnicity and age.

Methods: HIV diagnosis, mortality and census population data were used to derive lifetime risk estimates and 95% confidence intervals of HIV diagnosis among MSM by race/ethnicity and age. HIV diagnoses data reported to the National HIV Surveillance System (NHSS) by December 2022 were used. The numbers of HIV diagnoses (NHSS) and non-HIV deaths (National Center for Health Statistics mortality data) during 2017–2021 were used to calculate probabilities of a HIV diagnosis at a given age, conditional on never having received a HIV diagnosis prior to that age using a competing risks method. The lifetime risk estimate is the cumulative probability of HIV diagnosis from birth. Comparisons were made to findings from a 2010–2014 analysis. The analysis was conducted in DevCan 6.7.3.

Results: During 2017–2021, the lifetime risk of a HIV diagnosis among MSM was 1 in 7 overall. Lifetime risk among MSM was 1 in 3 for Black/African American persons, 1 in 5 for Hispanic/Latino persons, 1 in 7 for Native Hawaiian/other Pacific Islander persons, 1 in 11 for American Indian/Alaska Native persons and 1 in 15 for Asian persons and White persons. Lifetime risk improved for all races/ethnicities except for American Indian/Alaska Native, Hispanic/Latino and Native Hawaiian/other Pacific Islander MSM which stayed the same, compared to 2010–2014 (Table). For 10-year age-conditional risk of a HIV diagnosis, the highest 10-year risk experienced overall and for all races/ethnicities was at age 20, with risk decreasing with age. Compared to 2010–2014, improvements occurred for some but not all race/ethnicities. Estimating missed diagnoses in 2020 due to COVID-19, the unadjusted lifetime risk (14.60%) was 2.6% lower than the adjusted risk (14.98%) among MSM.

Conclusion: Overall, lifetime risk of HIV diagnosis improved among MSM, but this decrease was not seen across all races/ethnicities. The Ending the HIV Epidemic in the U.S. initiative is designed to scale up key HIV prevention and treatment strategies and is also working to address disparities. There is need for continued progress in HIV prevention and treatment since disparities persist by race/ethnicity among MSM.

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194 Quantifying HIV-1 Transmission Between People Who Inject Drugs and Other Populations in Kenya

Hanley Kingston1, Bhavna Chohan1, George-Nduwa2, Loice Mbogo3, Aliza Monroe-Wise1, Betsy Sambali1, Brandon Guthrie1, Sarah Masyukol1, David Bukusi3, John Scott1, Carey Farquhar4, Josh Herbeck4

1University of Washington, Seattle, WA, USA, 2Lund University, Lund, Sweden, 3Kenyatta National Hospital, Nairobi, Kenya, 4Bill and Melinda Gates Foundation, Seattle, WA, USA

Background: Although recent modeling suggests needle-syringe programs have reduced parental HIV transmission among people who inject drugs (PWID) in Kenya, the prevalence in this population remains high (~14–20%, compared to ~4% in the larger population). Reducing transmission or acquisition requires understanding historic and modern transmission trends, but the relationship between the PWID HIV sub-epidemic and the general epidemic in Kenya is not well understood. Incorporating 6-times more HIV sequences from PWID in Kenya than in prior studies, we quantified rates and direction of HIV-1 transmission involving PWID and other populations from the coast and Nairobi regions.

Methods: We aligned 303 new (2018-2021) HIV-1 pol sequences from PWID and their sexual and injecting partners with 2,666 previously published Kenyan sequences. We used genetic similarity cluster analysis (thresholds: patristic distance <0.045, aLRT >0.90) and maximum likelihood ancestral state reconstruction to estimate transmission histories at the population (female sex workers, men who have sex with men, PWID, or not key population) and regional (coast or Nairobi) levels. Transition counts estimate how often an ancestor sequence gave rise to a descendant sequence from a different population and/or region.

Results: In this cohort, 1,081 participants lived with HIV, of whom 274 (25%) were not virally suppressed and 303 (28%) had sequences available. Of new PWID sequences, 55% were in phylogenetic clusters, and the vast majority were interspersed with sequences from other key populations and from those not in key populations. Only 22% of clusters containing PWID sequences included a second PWID sequence. Ancestral state reconstruction (Figure 1) identified substantial transmission between the coast and Nairobi regions and more not-PWID to PWID transmission than PWID to PWID transmission.

Conclusion: Despite recruiting PWID from local injecting networks, we found minimal linked transmission in this population. This suggests low rates of recent parenteral transmission and supports interventions to reduce sexual transmission while maintaining needle-syringe programs. Because the epidemic among PWID and other populations are inter-related, interventions within the larger population, where we also observed the most transmission between regions, may have carry-over benefits for reducing HIV prevalence in PWID and vice versa. However, greater understanding of how PWID and non-PWID populations interact is needed.

195 Demographics Are Crucial to Interpret 95-95-95 Targets in African Populations With High ART Coverage

Andrea Brizzi1, Joseph Kagayi2, Robert Ssekubugu2, Alexandre Bienkimsop1, Méridie Monod3, Gertrude Nakigozi1, Larry W. Chang3, Thomas C. Quinn4, Fred Nalugoda5, Godfrey Kigozi6, Ronald M. Gallwitz7, Oliver Laeyendecker8, Mary Kate Grabowski9, Steven J. Reynolds10, Oliver Rattram11

1Institutions: Imperial College London, London, United Kingdom, 2Rakai Health Sciences Program, Kalisasa, Uganda, 3The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA

Background: Characterising the shifting dynamics of HIV unsuppressed populations in Africa is crucial to tailor cost-effective interventions necessary to end AIDS by 2030.

Methods: We analyzed HIV testing and viral load data collected between 2013 and 2019 from four consecutive surveys of the Rakai Community Cohort Study (RCCS), an open population census and HIV surveillance cohort in Uganda,
to estimate HIV seroprevalence and population viral load (VL) suppression over time by location, gender, and one-year age bands. Eligible participants were individuals aged 15 to 49 years old, resident in 40 communities under RCCS surveillance, four of which were hyperendemic Lake Victoria fishing communities. Surveys coincided with the implementation of Universal Test and Treat (UTT), starting in 2014 in fishing communities, and in 2017 elsewhere. All estimates were standardized to population level using census data and compared to UNAIDS 95-95-95 targets.

Results: Following UTT, viremia decreased from 4.9% (4.6–5.3) at baseline to 1.9% (1.7–2.2) in 2019 in inland communities and from 19.2% (18.0–20.4) at baseline to 4.7% (4.0–5.5) in 2019 in fishing communities. Crucially, reflecting population pyramids and the age and gender profile of HIV burden, population-level viral load did not concentrate in the age groups furthest from achieving UNAIDS 95-95-95 targets (Figure 1). For example, by 2019, in inland communities, women aged 15-19 and men aged 20-24 were furthest from achieving 95-95-95 targets but contributed only 4.7% (2.9-7.2) and 5.5% (3.8-7.8) to population-level viremia. In contrast, women aged 25-29 and men aged 30-34, who were close to or had achieved the 95-95-95 targets, each contributed approximately 10% to population-level viremia in 2019.

Conclusion: While 95-95-95 targets provide a useful benchmark for HIV control, they do not take into consideration the underlying population structures and may direct interventions towards groups which contribute marginally to the unsuppressed population. In this cohort, targeting men aged 25-34 rather than men aged 15-24 would result in larger reductions in the number of unsuppressed individuals, despite larger suppression rates in the former age-group.

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A New 2023–2024 Mpox Outbreak in Brazil: Lessons From a Reemerging Neglected Disease

Mayara Secco Torres da Silva, Carolina Coutinho, Thiago S. Torres, Amanda Echeverria-Guevara, Matheus O. Bastos, Pedro S. Martins, Maira B. Mesquita, Estevao P. Nunes, Ronaldo Moreira, Eduardo M. Peixoto, Edison E. Silva, Sandra Wagner-Cardoso, Valdilea Veloso, Beatriz Grinzmizt,1 for the INI-Fiocruz Mpox Study Group

1Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil; Oswaldo Cruz Foundation - Fiocruz, Rio de Janeiro, Brazil

Background: The 2022 mpox multi-national outbreak highly affected the Americas, with Brazil reaching 10,962 confirmed cases as of December 11th, 2022. After a peak in July–August, 2022, global mpox diagnoses decreased, and Brazil reported no cases between July and August, 2023 despite the absence of the mpox vaccine. We present initial evidence of an emerging and continuous mpox outbreak in Rio de Janeiro, Brazil, starting in September 2023.

Methods: We conducted a prospective cohort of participants (ppts) diagnosed with mpox (detectable MPXV PCR from any site) at a major infectious diseases’ referral center in Rio de Janeiro, Brazil. The data were analyzed as 1st outbreak (12th June 2022 – 31st May 2023) and 2nd/current outbreak (September 26th, 2023 – January 7th, 2024).

Results: We enrolled 417 ppts during the 1st outbreak, with no further mpox cases until the 2nd outbreak, when we enrolled 83 ppts (1 case of reinfection). Compared to the 1st outbreak, the 2nd/current outbreak presented higher proportions of cisgender men (96% vs 90%, p = 0.02) and men who have sex with men (MSM) (94% vs 81%, p = 0.01). There was an increased number of sexual partners (median 3 vs 2, p < 0.01) and reported anal sex (91% vs 68%, p < 0.01). Age, race and clinical characteristics were similar across the outbreaks. In the 2nd outbreak, most ppts took more than 5 days from symptoms onset to first assessment (71% in n = 58/82 vs 59.3% in n = 259/437, p = 0.05), and more ppt with mpox live with HIV (63% vs 51%, p = 0.05) (Table). Among PLHIV from the 2nd outbreak (n = 52), 5.9% had CD4 < 100 cells/mm³, 19% HIV-RNA viral load > 1,000 copies/mL, 9.6% concomitant opportunistic infections and 5.8% had suspected immune reconstitution inflammatory syndrome. During the 2nd outbreak, ppts showed a high frequency of concomitant bacterial STI (36%, n = 26/72) and HCV past/current infection (11%, n = 9/83). The hospitalization rate was 12% (n = 10/83) and as of January 7th, 2024, 18 ppts were still under follow-up.

Conclusion: This marks the inaugural report of a recent mpox outbreak in Brazil, following a period without diagnosed cases, once again disproportionately affecting MSM and PLHIV individuals. Our findings suggest ongoing unnoticed community mpox transmission in Rio de Janeiro, Brazil, emphasizing the crucial need to enhance surveillance strategies to promptly identify emergent STIs in the context of HIV care and prevention services. Vaccines should be made available in LMIC to prevent new outbreaks.
Together, campaign implemented marketing on platforms including social media, dating apps, and search and display advertising. Marketing was primarily to men who have sex with men (MSM), especially Black and Hispanic MSM, Black women, and transgender women. Building Healthy Online Communities developed messages and in-app buttons in partnership with dating apps including Grindr and BLK. Persons ages 15+ in the US and Puerto Rico were eligible to order 1-2 HIV self-tests every 90 days. Ordering wasn’t restricted by prior HIV diagnosis or PrEP usage, but persons reporting ARV use were encouraged to order HIV self-tests to others. A short survey was offered post-order with an opt-in for follow-up surveys. Ten- and 60-day follow-up surveys on their HIV self-test experience were conducted.

Results: In March 2023, TTMH launched, with 181,558 orders placed in the first 9 months. Most orders (86%) were for two tests, with 337,812 total tests distributed. Most participants (109,956, 62%) came from the Grindr app. Sixty percent (108,715) of all orders contained enough information to describe participants in terms of the priority populations. Of these 61% were from men reporting male partners in the past 12 months (18% from Black MSM and 33% from Hispanic MSM), 10.7% from gender diverse persons, and 10% from Black women. Most orders (26%) were placed by persons who had never tested for HIV, or who had last tested >12 months ago (27%). Over half of participants, 86,143 (56.5%) opted into follow-up communications and as of December 11, 2023, 5,294 (6.1%) completed the 10-day survey. Among them, 109 (2.1%) reported a positive result with the HIV self-test, 6.5% sought additional STI testing, and 4.5% self-reported starting PrEP after receiving the self-test.

Conclusion: Overall, the TTMH program has very high demand, with many persons from priority populations accessing HIV testing for the first time. Many sought additional clinical services after HIV self-testing. It is important for clinicians to be aware of the demand for HIV self-testing and how it may fit into their patient care, including preparing for discussions about HIV follow-up testing, pre-exposure prophylaxis and treatment.

Incidence of Health Facility Switching and HIV Viral Rebound in Uganda: A Population-Based Study

Joseph G. Rosen,1 Anthony Ndayizabanda,2 Ronald M. Galimangwa,2 Robert Sebugwubi2,3, Katherine Rucinski,2 Gertrude Nakigaz1, Fred Nakiguda,3 Godfrey Kigozi,3 Thomas C. Quinn,4 Larry W. Chang,5 Caitlin E. Kennedy,6 Steven J. Reynolds,7 Joseph Kegaya,3 Mary Kate Grabowski,1,8,9 for the Rakai Health Sciences Program

1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2Rakai Health Sciences Program, Kalisizo, Uganda, 3The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

Background: Prior studies have shown that a sizeable fraction of persons on antiretroviral therapy (ART) in Africa, once believed to be care-disengaged, have actually transferred to other healthcare facilities for continued HIV treatment. However, the relationship between facility switching and virion outcomes, specifically HIV viral rebound, among persons on ART is poorly understood.

Methods: We used population-level data collected between 2015 and 2020 from 40 continuously surveilled communities, including four hyperendemic Lake Victoria fish landing sites, in the Rakai Community Cohort Study. Persons aged 15-49 years with serologically confirmed HIV infection self-reporting current ART use and contributing ≥2 follow-up visits were included in the study. Facility switching and virologic outcomes were assessed between two consecutive study visits (i.e., index and follow-up visits, ~18-month visit interval). Persons attending different HIV treatment facilities between index and follow-up visits were classified as having switched facilities. The primary outcome was laboratory-confirmed viral rebound, defined as ≥200 HIV RNA copies/mL at follow-up visit among individuals exhibiting viral load suppression (<200 copies/mL initially). Multivariable Poisson regression with generalized estimating equations and robust standard errors was used to model associations between facility switching and viral rebound, reported as adjusted incidence rate ratios (aIRR) with 95% confidence intervals (95%CI).

Results: Overall, 2,257 persons self-reporting current ART use (median age: 35 years, 65% women, 92% virally suppressed at index visit) contributed 3,335 visit-pairs and 5,959 person-years (py) to the analysis. Facility switching was common (4.8 switches per 100 py, 95%CI: 4.2-5.5) and highest in persons aged 15-29 years (7.3 switches per 100 py, 95%CI: 5.9-9.1), fishing community residents (7.4 switches per 100 py, 95%CI: 6.3-8.6), and in-migrants (10.4 switches per 100 py, 95%CI: 8.3-13.1). Among initially suppressed persons (n=2,076), the incidence of viral rebound was over twice as high in persons switching facilities relative to those attending the same clinic over the visit-interval (aIRR 2.27, 95%CI: 1.16-4.45).

Conclusion: Facility switching was common and associated with viral rebound among initially suppressed persons. Investments in more agile, person-centered HIV care models for mobile clients are needed to address system inefficiencies and bottlenecks that can disrupt HIV treatment continuity.

Lakeside Combined HIV and Schistosomiasis Services in Malawian Fishermen: A Cluster Randomized Trial

Augustine T. Choko,1 Kathryn L. Dover,1 Sekeleghe A. Kayuni,1 Donaldson Converse1, Anthony Butterworth1, Amaya Bustinduy1, J. Russel Stothard2, Wala Ramchedza2, Madalo Mukoka-Thindwa3,1 James Jafali,1 Peter MacPherson1, Katherine Fielding1, Nicola Desmond1, Elizabeth L. Corbet3

1Malawi-Liverpool Welcome Trust Clinical Research Programme, Blantyre, Malawi, 2University of California Los Angeles, Los Angeles, CA, USA, 3George Washington University, Washington, DC, USA, 4London School of Hygiene & Tropical Medicine, London, United Kingdom, 5Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 6University of Glasgow, Glasgow, United Kingdom, 7London School of Hygiene & Tropical Medicine, Blantyre, Malawi

Background: Undiagnosed HIV and Schistosomiasis are highly prevalent among fishermen in the Great Lakes region of Africa. Combined interventions to address barriers to diagnosis, treatment and prevention for both infections are urgently needed.

Methods: Between March 2022-January 2023, we conducted a cluster-randomized trial with 45 lakeside “boat-team” (clusters) in Lake Malawi. Clusters were randomly allocated (1:1:1) to: 1) an enhanced standard of care (SOC), with beach clinics offering HIV testing and referral, and schistosomiasis presumptive treatment (praziquantel) advertised by leafletting; 2) peer-educator (PE), with a peer-nominated boat crew member trained to promote beach clinic services; or 3) peer-distributor-educator (PDE), where PEs distributed oral HIV self-test (HTVST) kits in addition to promoting beach clinic services. The co-primary outcomes (measured at 28 days) were: composite self-reported ART initiation, or booked for voluntary male medical circumcision (VMMC); and 1 S. haematobium egg seen on light microscopy of the filtrate from 10mls urine (“egg-positive”). Secondary outcomes included self-reported HIV testing in the PDE arm, and observed HIV testing in eSOC and PE arms as well as perceived acceptability of HIV pre-exposure prophylaxis (PrEP). Analyses were by intention-to-treat with multiple imputation while accounting for clustering and any baseline imbalance.

Results: Of 6036 fishermen screened, 5207 (86.3%) were eligible: (SOC: 1745 [87.6%]; PE: 1687 [81.9%]; PDE: 1775 [89.5%]). Participant characteristics were well balanced by intention-to-treat with multiple imputation while accounting for clustering and any baseline imbalance.

Conclusion: Facility switching was common and associated with viral rebound among initially suppressed persons. Investments in more agile, person-centered HIV care models for mobile clients are needed to address system inefficiencies and bottlenecks that can disrupt HIV treatment continuity.
203 Improving Posthospital Outcomes in People With HIV: A Multicenter Randomized Trial in Tanzania

Robert Peck1, Benson Issarow1, Godfrey Kigisa1, Elialilia Okello1, Severin A. Kakabaka2, Robert Peck1, Benson Issarow1, Godfrey Kigisa1, Elialilia Okello1, Severin A. Kakabaka2, Robert Peck1, Benson Issarow1, Godfrey Kigisa1, Elialilia Okello1, Severin A. Kakabaka2, Robert Peck1, Benson Issarow1, Godfrey Kigisa1, Elialilia Okello1, Severin A. Kakabaka2, Robert Peck1, Benson Issarow1, Godfrey Kigisa1, Elialilia Okello1, Severin A. Kakabaka2

Background: In spite of the widespread availability of antiretroviral therapy (ART), people living with HIV (PLWH) still experience poor outcomes with high mortality during and after hospital admissions. Delayed linkage to HIV care after hospital discharge is a major risk factor. We tested a linkage case management intervention (“Daraja” = “Bridge” in Kiswahili) to address barriers to HIV care engagement after hospital discharge.

Methods: We conducted a single-blind, individually randomized trial to evaluate the effectiveness of the Daraja intervention (NCT03858998). PLWH who were either ART-naïve or ART defaulters were recruited from 20 hospitals in northern Tanzania. Participants were randomized before hospital discharge to receive either the Daraja intervention or standard of care. The Daraja intervention consisted of 5 sessions conducted by a social worker over a 3 month period. The primary outcome of all-cause mortality at 12 months was confirmed by death certificates, hospital records, or verbal autopsies. Secondary outcomes related to HIV clinic attendance, ART use, and viral load suppression were extracted from HIV medical records.

Results: We enrolled 500 hospitalized PLWH between March 2019 and February 2022. The mean age was 37 years, 77% were female, 35% had CD4 counts <100 cells/μL, 75% were ART naïve, and characteristics were similar between arms. Intervention uptake was high with 86% of expected sessions successfully completed; 496 participants completed 12 months of follow-up (2 withdrew consent; 2 lost-to-follow-up). Eighty-five (17%) participants died; mortality did not differ by study arm (43 vs. 42 deaths, p=0.96). Half of deaths occurred within 30 days after discharge. By contrast, the Daraja intervention reduced time to HIV clinic attendance and ART initiation (p<0.0001).

Intervention participants also achieved higher rates of ART adherence (81% vs. 68%, p=0.002) and viral load suppression at 12 months (75% vs. 67%, p=0.001). Retention at 24 and 36 months was defined as attending a visit at 13-24 months, 25-36 months and 37-48 months after ART initiation. 12, 24 and 36 month retention was defined as attending a visit at 13-24 months, 25-36 months and 37-48 months after ART initiation, respectively. Outcomes after 12 months were conditional on being retained in the previous period.

Results: 59,118 clients (67% female, 17% age 18-24) initiated ART during the study period. Proportion enrolled in DSD was 2% (N=1,431), 23% (N=7,238), 37% (7,845) by 12, 24 and 36 months; eligible but not enrolled was 12% (N=7,339), 48% (N=15,087), 46% (N=9,761) by 12, 24 and 36 months. Retention and viral suppression at 12 months were lower for those in DSD vs eligible. Retention at 24 and 36 months was higher for those in DSD vs eligible (relative risk: 1.18 [95% confidence interval 1.07-1.31]). Viral suppression remained lower than those with a VL at 24 and 36 months was similar between those in DSD vs eligible. There were no differences in outcomes by gender or age group.

Conclusion: South African DSD clients had higher retention and similar viral suppression at 24 and 36 months after ART initiation. The observed decrement to outcomes for DSD clients at 12 months requires further examination, particularly as new guidelines allowing DSD enrollment ≥4 months on ART. There was a missed opportunity for DSD enrollment as most of those eligible were not enrolled ≤3 years on ART.

204 What Are the Outcomes for Established Clients Enrolled in DSD During the First 3 Years on ART?

Amy N. Huber1, Lise Jamieson1, Musa Manganye1, Lufuno Malalu2, Thato Chidarikire3, Matthew P. Fox4, Amy N. Huber1, Lise Jamieson1, Musa Manganye1, Lufuno Malalu2, Thato Chidarikire3, Matthew P. Fox4, Amy N. Huber1, Lise Jamieson1, Musa Manganye1, Lufuno Malalu2, Thato Chidarikire3, Matthew P. Fox4

Background: Replacing routine clinic visits with differentiated service delivery (DSD) models for HIV treatment could benefit DSD clients and the health system, but its value depends on maintaining or improving patient outcomes. South Africa’s DSD models include facility pick-up points, external pick-up points and adherence clubs, which facilitate easier access to medications. We conducted a prospective record review to compare outcomes of DSD clients to those eligible but not enrolled in DSD in South Africa.

Methods: Among adults initiating ART between 2016-2021 at 18 primary healthcare facilities, we compared retention and viral suppression for DSD clients to those DSD eligible but not enrolled using TIER.Net records. For reference we also included those not DSD eligible. DSD eligibility was defined per guidelines (2016-2019: 2 suppressed viral load (VL) (<400c/mL) and ≥12 months ART; 2020-2021: 1 suppressed VL (<350c/mL) and ≥6 months ART). DSD enrollment was defined as any DSD interaction in the previous 12 months before the 12, 24, or 36 month time point. DSD eligibility and enrollment were reclassified every 12 months. Outcomes were assessed at 12, 24, and 36 months after ART initiation. 12, 24 and 36 month retention was defined as attending a visit 13-24 months, 25-36 months and 37-48 months after ART initiation, respectively. Outcomes after 12 months were conditional on being retained in the previous period.

Results: 37% (7,845) by 12, 24 and 36 months; eligible but not enrolled was 12% (N=7,339), 48% (N=15,087), 46% (N=9,761) by 12, 24 and 36 months. Retention and viral suppression at 12 months were lower for those in DSD vs eligible. Retention at 24 and 36 months was higher for those in DSD vs eligible (relative risk: 1.18 [95% confidence interval 1.07-1.31]). Viral suppression remained lower than those with a VL at 24 and 36 months was similar between those in DSD vs eligible. There were no differences in outcomes by gender or age group.

Conclusion: South African DSD clients had higher retention and similar viral suppression at 24 and 36 months after ART initiation. The observed decrement to outcomes for DSD clients at 12 months requires further examination, particularly as new guidelines allowing DSD enrollment ≥4 months on ART. There was a missed opportunity for DSD enrollment as most of those eligible were not enrolled ≤3 years on ART.

205 Opt-Out HIV Testing in Emergency Departments Successfully Addresses Key Gaps in Testing

Lisa Hamzah1, Kathryn Childs1, Larissa Mulka1, Elialilia Okello1, Benson Issarow1, Sydney Rosen2, Steven Kegg1, Lisa Rosen-Metsch1, Myung Hee Lee3, Lewis Fox4, Amy N. Huber1, Lise Jamieson1, Musa Manganye1, Lufuno Malalu2, Thato Chidarikire3, Matthew P. Fox4, Lisa Hamzah1, Kathryn Childs1, Larissa Mulka1, Elialilia Okello1, Benson Issarow1, Sydney Rosen2, Steven Kegg1, Lisa Rosen-Metsch1, Myung Hee Lee3, Lewis Fox4, Amy N. Huber1, Lise Jamieson1, Musa Manganye1, Lufuno Malalu2, Thato Chidarikire3, Matthew P. Fox4

Background: Improving posthospital care in the global effort to get to zero AIDS-related deaths. Delayed linkage to HIV care after hospital discharge is a major risk factor. We tested a linkage case management intervention (“Daraja” = “Bridge” in Kiswahili) to address barriers to HIV care engagement after hospital discharge.
206 Few Discordant HIV Ag/Ab and RNA Test Results Among Persons in a National Cohort of PrEP Users

Weiming Zhu, Ya-Lin A. Huang, Kevin P. Delaney, Rupa Patel, Athena Kourtis, Karen W. Hoover

Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: PrEP users can have ambiguous HIV test results for detection of primary infection. The CDC 2021 PrEP guidelines recommend HIV RNA testing for PrEP initiation and monitoring. We evaluated occurrence of discordant HIV test results in oral and long-acting cabotegravir (CAB-LA) PrEP users in a large real-world cohort of PrEP users.

Methods: We analyzed the HealthVerity HIV cohort with linked longitudinal medical claims, antiretroviral prescriptions, and laboratory testing records. Our primary outcomes were discordant HIV Ag/Ab and RNA test results, new HIV diagnoses, and long-acting Early Viral Inhibition (LEVI) cases among PrEP users. We used a validated algorithm and identified persons prescribed oral or injectable PrEP and extracted all their laboratory records. We defined a “combined test” as both Ag/Ab and RNA testing within 7 days. We analyzed all tests from 30 days before the first PrEP prescription through 30 days after last prescription. Among persons with discordant results, we reviewed all their laboratory tests, diagnosis records, and ARV prescriptions.

Results: Among 30,548 PrEP users, we identified 9,090 combined tests in 5,391 individuals; 8995 (99.0%) were same-day tests. HIV Ag/Ab and RNA results were concordant for 9070 (99.8%) tests with 18 dual positives (+) in 17 persons, and 9052 dual negatives (-) in 5374 persons. We identified 14 combined tests in 12 persons with Ag/Ab(-) and RNA(+) results, accounting for 0.15% of all combined tests. Review of records found 4 HIV diagnoses; 4 likely false positive RNA tests (with repeated dual -) tests and follow-up >30 days); and 4 inconclusive results (insufficient follow-up) (Figure). Excluding inconclusive cases, RNA tests had a false positive rate (FPR) of 0.04% and a positive predictive value (PPV) for HIV infection of 84% among PrEP users. No LEVI cases were observed among 439 CAB-LA users. We also found 6 discordant tests in 4 patients with Ag/Ab(-) and RNA(+) results. Of these, one person had a likely false positive Ag/Ab test (FPR of 0.01%, PPV 94.4%), while 3 persons had insufficient follow-up.

Conclusion: Discordant combined test results among PrEP users were rare and a third of discordant tests identified a new HIV diagnosis, accounting for 19% of all diagnosed HIV infections. Combined testing resulted in similar early HIV diagnoses rates and potential false positive NAT rates. The FPR of Ag/Ab testing was low. Further assessment of predictive values and cost-effectiveness of HIV RNA testing of PrEP users is warranted.
of penicillin >30 days before delivery, and 15 of 31 (48%) had >30 days of RPR titer decrease. Two congenital syphilis cases were identified (RPR titer 4x mother’s RPR; 6.4% vertical transmission and 0.4% population prevalence) both with maternal diagnosis and treatment <30 days before delivery. Age-adjusted risk factors associated with maternal syphilis included younger age, reporting both sex partners, experiencing recent intimate partner violence and alcohol use (Table).

Conclusion: These novel data demonstrate a remarkably high occurrence of syphilis in pregnancy among women enrolled in antenatal PrEP services, with half of women not fully treated in pregnancy leading to preventable congenital syphilis. There is a clear and urgent need to integrate syphilis prevention and treatment into antenatal PrEP services.

### Table 1. Baseline demographics and health characteristics among pregnant women on oral PrEP tested for syphilis during pregnancy in Cape Town, South Africa [March 2022–December 2022]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (N=308)</th>
<th>Women on ISL+LEN (N=154)</th>
<th>Women on B/F/TAF (N=154)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25 (21–31)</td>
<td>26 (21–31)</td>
<td>24 (20–29)</td>
</tr>
<tr>
<td>Gender</td>
<td>64% male</td>
<td>65% male</td>
<td>63% male</td>
</tr>
<tr>
<td>Education</td>
<td>11% primary</td>
<td>11% primary</td>
<td>11% primary</td>
</tr>
<tr>
<td>Income</td>
<td>32% low</td>
<td>32% low</td>
<td>32% low</td>
</tr>
<tr>
<td>Occupation</td>
<td>38% student</td>
<td>36% student</td>
<td>40% student</td>
</tr>
<tr>
<td>Religion</td>
<td>56% Christianity</td>
<td>57% Christianity</td>
<td>55% Christianity</td>
</tr>
</tbody>
</table>

No unexpected safety issues were observed.

### Efficacy and Safety of Weekly Istratavir Plus Lenacapavir in PWH at 24 Weeks: A Phase II Study

Amy Colson*, Gordon Crofoot†, Peter J. Ruane*, Moti Ramgopal*, Alexandra W. Dettler*, Ronald G. Nahass*, Gary Sinclair†, Megebe Beke†, Chris Deaton†, Angelina S. Liu*, Eea Mortensen*, Martin S. Rhee†, Elizabeth G. Rhee†, Jared Baeten†, Joseph E. Eron‡

*Community Resource Initiative, Boston, MA, USA; †Cohort Research Center, Houston, TX, USA; ‡University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Istratavir (ISL), a nucleoside reverse transcriptase translocation inhibitor, and lenacapavir (LEN), a C31 inhibitor have potent anti-HIV-1 activity and pharmacokinetic profiles permitting once-weekly (QW) oral dosing. We investigated efficacy and safety of ISL+LEN in virologically suppressed people with HIV-1.

Methods: In this Phase 2, randomized, open-label, active-controlled study (NCT05052996), virologically suppressed adults on bitracegravir/erabbitinib/tenofovir alafenamide fumarate (B/F/TAF) were randomized to either oral ISL 2 mg + LEN 300 mg QW or to continue daily B/F/TAF. The primary efficacy endpoint was the proportion of participants with HIV-1 RNA ≥ 50 copies/mL (FDA-defined Snapshot algorithm) at Week 24 (W24). Safety parameters, including CD4+ T-cell and absolute lymphocyte counts (ALC) and adverse events (AEs), were also evaluated.

Results: A total of 104 participants were randomized and dosed (52/group); median age (range) was 40 (26–76) years, and 19 (18.3%) were female at birth. A total of 104 participants were randomized and dosed (52/group); median age (range) was 40 (26–76) years, and 19 (18.3%) were female at birth. A total of 104 participants were randomized and dosed (52/group); median age (range) was 40 (26–76) years, and 19 (18.3%) were female at birth. A total of 104 participants were randomized and dosed (52/group); median age (range) was 40 (26–76) years, and 19 (18.3%) were female at birth. A total of 104 participants were randomized and dosed (52/group); median age (range) was 40 (26–76) years, and 19 (18.3%) were female at birth.

Conclusion: In this Phase 2 study, the first QW oral ARV regimen of ISL+LEN maintained viral suppression at W24 and was well tolerated. The ISL 2 mg dose showed no clinically significant decreases in CD4+ T-cell counts or ALCs as seen previously with higher daily, weekly, and monthly doses of ISL.

### HepB-CgP Vaccine Is Superior to HepB-alum in People With HIV and Prior Vaccine Nonresponse: A5379

Kristen Marks*, Minhee Kang†, Trinh Umbuja‡, Andrea Cox†, Karen J. Vigil‡, Ngan T. Ta*, Ayotunde Omoz-Darhe*, Jennifer C. Price‡, Josaphat Rokigel§, Leolin Katsidzira*, Hugo Perazzo*, Kevin Knowles**, Beverly L. Alston-Smith†, Kenneth E. Sherman‖, for the ACTG A5379 (Bee-HIVE) Study Team

*Well Cornell Medicine, New York, NY, USA; †Harvard TH Chan School of Public Health, Boston, MA, USA; §The Johns Hopkins University School of Medicine, Baltimore, MD, USA; ‡Boots Was University of Houston, Houston, TX, USA; §Hanoi Medical University, Hanoi, Vietnam; ‖Bootsman Harvard AIDS Institute Partnership, Cambridge, Boston; ‡University of California San Francisco, San Francisco, CA, USA; †Walter Reed Project–Kenya, Nairobi, Kenya

Background: Conventional Hepatitis B surface antigen (HBsAg)-based vaccines achieve seroprotection response (SPR, HBsAb >10 mIU/mL) in 35–80% of people with HIV (PWH) with 3 doses. HepB-CgP vaccine (with a TLR-9 agonist adjuvant) achieves high SPR in PWH, but limited data exist for non-responders to conventional vaccines.

Methods: ACTG A5379 is an ongoing, open-label study to evaluate immunogenicity of HepB-CgP in PWH. Prior vaccine non-responders were on ART with CD4 ≥ 100 cells/mm³ and HIV-1 RNA <100 copies/mL without past or present serologic evidence of HBV or HIV vaccine response. Participants were randomized 1:1:1 to: 2 doses of HepB-CgP intramuscularly (IM) (20 mcg recombinant HBsAg, 3000 mcg Cpg 1018® adjuvant) at Wks 0 and 4 (2-CgP); 3 doses of HepB-CgP IM at Wks 0, 4, 24 (3-CgP); or 3 doses of HepB-alum IM (20 mcg recombinant HBsAg) at Wks 0, 4, 24 (3-alum). Primary SPR was defined at Wk 12 for 2-CgP and Wk 28 for 3-CgP and 3-alum. We assessed noninferiority (NI) of 2-CgP vs 3-alum with a 10% margin and superiority of 3-CgP vs 3-alum. Predefined primary analysis set excluded missed sample collection. Safety was also assessed.

Results: Of the 561 eligible participants enrolled at 41 sites from 10 countries: 64% were male, 42% Black, 35% White, 17% Asian, 22% Hispanic. Median age was 46 years (range 18–70), 56% enrolled in the US, 21% Africa, 17% Asia, 6% S. America. Median CD4 was 638 cells/mm³; 94% had HIV-1 RNA <40 copies/mL, 29% BMI >30, and 13% diabetes. 96% completed all prescribed doses. The analysis included 505 participants (99% of 508 in the primary analysis set). SPR was achieved in 93% of 2-CgP (n=174), 99% of 3-CgP (n=169), and 80% of 3-alum (n=162). SPR difference between 2-CgP and 3-alum was 13% (97.5% CI: 5%, 22%), achieving NI and indicating superiority. SPR of 3-CgP was superior to 3-alum with a difference of 19% (97.5% CI: 11%, 27%), achieving NI and indicating superiority. SPR of 3-CgP vs 3-alum was 13% with a 10% margin and superiority of 3-CgP vs 3-alum. Predefined primary analysis set excluded missed sample collection. Safety was also assessed.

Conclusion: In this study of PWH with prior vaccine non-response, both 2 and 3 doses of HepB-CgP achieved superior SPR compared to 3 doses of HepB-alum. No unexpected safety issues were observed.
Efficacy, Safety, and Immunogenicity of H56:IC31 Vaccine for Prevention of Recurrent TB

Alvaro Borges1, Marisa Russel1, Dereck Tait1, Elana van Brakel1, Andrea Cabibbe1, Daniela Cirillo1, Elisa Nemers1, Thomas Scriba1, Gavin Churcyhard1, Rodney Dawson1, Isa Sabi1, Andreas H. Diacen1, Rasmus Mortensen1, Mark Hatthelen2, for the POR TB consortium

1Staters Serum Institut, Copenhagen, Denmark, 2International AIDS Vaccine Initiative, Cape Town, South Africa, 3RICS/Ospedale San Raffaele, Milano, Italy, 4South African Tuberculosis Vaccine Initiative, Cape Town, South Africa, 5The Aurum Institute, Johannesburg, South Africa, 6University of Cape Town, Cape Town, South Africa, 7Mbeya Medical Research Center, Mbeya, United Republic of Tanzania, 8TASK Applied Science, Cape Town, South Africa

Background: Persons with tuberculosis (TB) who are deemed cured on completion of treatment remain at higher risk of recurrent disease. The TB vaccine candidate H56:IC31 has been shown to be safe and immunogenic in phase 1/2 studies, including in treated TB patients. Whether H56:IC31 can reduce the risk of recurrent TB is unknown.

Methods: In a multicenter, double-blind, randomized, placebo-controlled, event-driven trial in South Africa and Tanzania, we enrolled participants aged 18-60 years, without HIV, who were sputum smear-negative upon completion of treatment for drug-sensitive pulmonary TB. Participants were randomly assigned (1:1) to receive two doses of H56:IC31 or placebo (56 days apart) and followed up for 1 year. The primary endpoint was recurrence of culture-confirmed pulmonary TB. Vaccine efficacy (VE) estimates with 95% confidence interval (95%CI) were derived from Cox proportional hazard models. Secondary endpoints included TB relapse or reinfection as differentiated by whole genome sequencing of paired sputum samples, safety, and immunogenicity.

Results: 831 participants (mean age 34.7 years, 27.6% female; 66.1% black African; 76% from South Africa) were enrolled; 415 received H56:IC31 and 416 placebo. In the primary analysis, recurrent TB was observed in 23 (12 relapse; 11 reinfection) of 416 participants in the placebo group and 14 (6 relapse; 8 reinfection; 1 indeterminate) of 406 participants (3.4%) in the placebo group. VE for recurrence was -73.8% (95%CI: -246.9 to 9.8%; P=0.10). VE for relapse was -116.1% (-522.2 to 16.3%; P<0.01) and for reinfection VE was -21.1% (-245.3 to 56.5%; P=0.71). Participants in the H56:IC31 group reported more mild-to-moderate local injection reactions than in the placebo group. No H56:IC31-related serious adverse events were observed. Participants receiving H56:IC31 mounted robust H56-specific CD4+ T cell responses and H56-specific humoral (serum IgG) responses.

Conclusion: This is the first reported trial with a prevention of recurrent TB design. Vaccination with H56:IC31 upon treatment completion for pulmonary tuberculosis did not reduce the risk of recurrent tuberculosis. H56:IC31 was well-tolerated and immunogenic, but may have increased the risk of relapse by endogenous strains.

Efficacy, Safety, and PK of BIC/FTC/TAF in Adults With HIV and Tuberculosis on Rifampicin at Week 24

Anushka Naidoo1, Kogieleum Naidoo1, Marothi P. Letsoalo1, Hylke Waelwijn1, Gillian Dorse1, Rubeshan Perumal1, Mahomed-Yunesu S. Moosa1, Emmanuelle C. Osuala1, Resha Boodhram1, Dennis Israelshi1, Pablo Denti1, James F. Rooney1, Kelly Dooley1, for the INSIGHT Trial Team

1Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, 2University of Cape Town, Cape Town, South Africa, 3University of KwaZulu-Natal, Durban, South Africa, 4Gilead Sciences, Inc, Foster City, CA, USA, 5Vanderbilt University, Nashville, TN, USA

Background: Integrase strand transfer inhibitors, bicinegravir (BIC) and dolutegravir (DTG), are currently recommended for the treatment of HIV. However, the efficacy, safety, and pharmacokinetics (PK) of BIC in people with HIV (PWH) and tuberculosis (TB) taking rifampicin-based therapy has not been evaluated.

Methods: INSIGHT (NCT04734652) is an open-label, non-comparative, phase-2b randomised controlled trial in ART-naïve or non-naïve adults with HIV (CD4+ >50 cells/μL) and TB, taking a rifampicin-based TB regimen (for ≤ 8 weeks). Participants were randomised 2:1 to the BIC arm (bicinegravir- emtricitabine (FTC)/tenofovir alafenamide (TAF)) or a standard of care DTG arm (tenofovir, lamivudine, dolutegravir (TLD)), with BIC/FTC/TAF or DTG dosed twice daily, until 2 weeks post-TB treatment and once daily thereafter, until 48 weeks. Participants underwent regular clinical and safety visits, including HIV viral load measurements at baseline and weeks 4, 8, 12, 24, 40 and 48. Semi-intensive PK sampling was performed during TB treatment and post-TB treatment. Non-comparitional PK analyses for BIC were conducted in R using the PKINCA package (version 10.2). We report preliminary endpoint results for the proportion of participants with plasma HIV-1 RNA <50 copies/mL at week 24.

Results: We enrolled 122 participants; 80 in the BIC and 42 in the DTG arm. Overall, 43 (35%) were female, with median (IQR) baseline viral load (copies/mL) and CD4+ (cells/μL) at week 24 was 257 (197-485) (BIC arm) and 231 (170-311) (DTG arm). HIV-1 RNA at week 24 was <50 copies/mL in 71/73 (97%) and 36/37 (97%) of participants in the BIC and DTG arms, respectively, in the per-protocol analysis (Figure 1). HIV-1 RNA was absent in all participants in both arms at week 48.

Conclusion: Data from INSIGHT suggest that twice daily bicinegravir- emtricitabine/tenofovir-alafenamide is effective in PWH with TB taking rifampicin-based treatment. Safety, PK, and virologic response data support the use of this regimen in PWH and TB.

Long-Acting Injectable CAB/RPV Is Superior to Oral ART in PWH With Adherence Challenges: ACTG A5359

Aadia I. Rana1, Yajing Bao2, Lu Zheng3, Sara Sieczkarski4, Jordan E. Lake4, Carl J. Fichtenbaum5, Tia Morton6, Lawrence Fox7, Paul Wannamaker8, Jose R. Castillo-Manchilla9, Kati Vandermeulen10, Chancelle Wimbish11, Karen T. Tashima11, Raphael J. Landovitz11, for the ACTG A5359 Team

1University of Alabama at Birmingham, Birmingham, AL, USA, 2Harvard T.H. Chan School of Public Health, Boston, MA, USA, 3Frontier Science & Technology Research Foundation, Inc, Amherst, NY, USA, 4University of Texas at Houston, Houston, TX, USA, 5University of Cincinnati, Cincinnati, OH, USA, 6National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 7VII+Healthcare, Research Triangle Park, NC, USA, 8INSIGHT (NCT04734652) is an open-label, non-comparative, phase-2b randomised controlled trial in ART-naïve or non-naïve adults with HIV (CD4+ >50 cells/μL) and TB, taking a rifampicin-based TB regimen (for ≤ 8 weeks). Participants were randomised 2:1 to the BIC arm (bicinegravir- emtricitabine (FTC)/tenofovir alafenamide (TAF)) or a standard of care DTG arm (tenofovir, lamivudine, dolutegravir (TLD)), with BIC/FTC/TAF or DTG dosed twice daily, until 2 weeks post-TB treatment and once daily thereafter, until 48 weeks. Participants underwent regular clinical and safety visits, including HIV viral load measurements at baseline and weeks 4, 8, 12, 24, 40 and 48. Semi-intensive PK sampling was performed during TB treatment and post-TB treatment. Non-comparitional PK analyses for BIC were conducted in R using the PKINCA package (version 10.2). We report preliminary endpoint results for the proportion of participants with plasma HIV-1 RNA <50 copies/mL at week 24.

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Conclusion: Data from INSIGHT suggest that twice daily bicinegravir- emtricitabine/tenofovir-alafenamide is effective in PWH with TB taking rifampicin-based treatment. Safety, PK, and virologic response data support the use of this regimen in PWH and TB.
RNA ≤ 200 c/mL in Step 1 were randomized to monthly LAI (with/without oral lead-in) vs. continuation of SOC ART for 52 weeks (Step 2). Primary composite endpoint was the earliest occurrence of virologic failure (VF, confirmed HIV-1 RNA > 200 c/mL) or treatment discontinuation. Key secondary efficacy endpoints included VF, treatment-related failure (VF or discontinuation due to adverse events, AEs) and treatment discontinuation. On Feb 12, 2024 a pre-planned interim review by an independent Data and Safety Monitoring Board recommended to stop randomization and offer LAI to all eligible participants. We present the interim results on which the DSMB recommendation was made.

**Results:** As of 3 Jan 2024, 434 eligible participants were enrolled in Step 1. Median age 40 years, 70% male, 64% Black/African American, 17% Hispanic, 5% transgender, 14% current/prior injection drug use, median CD4+ cells 270/mm³, and median HIV-1 RNA 3.55 log₁₀ c/mL. 294 eligible participants were randomized in Step 2 (LAI n=146, SOC n=148). Cumulative probability of AEs was similar in both arms. Three participants on LAI had ≥ Grade 3 injection site reactions (ISR) and one discontinued due to ISR. All efficacy endpoints favored the LAI arm (Table). Although the primary endpoint did not meet the predefined stopping criterion for this interim analysis (nominal 98.75% confidence interval excluding zero), key secondary endpoints of VF and treatment related failure met this stringent criterion, demonstrating superiority of the LAI arm vs. SOC. Two confirmed VFs in each arm had new resistance associated mutations (RAMS) including ≥ 2 new integrase inhibitor RAMs in both LAI participants.

**Conclusion:** When considering all endpoints together, long-acting CAB/RPV demonstrated superior efficacy compared to daily oral SOC in PWH with adherence challenges.

### Table: Kaplan-Meier cumulative probabilities for primary and key secondary endpoints and difference in probabilities between LAI and SOC arms

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>CAD-LAI/RPV LA</th>
<th>SOC (n=148)</th>
<th>Difference (nominal 95.75% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary: Treatment failure (VF or discontinuation)</td>
<td>28/288 (10%)</td>
<td>41/288 (14%)</td>
<td>-4.4% (90.9%, 0.0%)</td>
</tr>
<tr>
<td>Secondary: Virologic failure</td>
<td>11/288 (3.8%)</td>
<td>20/288 (7.0%)</td>
<td>-3.2% (91.7%, -5.4%)</td>
</tr>
<tr>
<td>Secondary: Treatment-related failure (VF or discontinuation due to AEs)</td>
<td>9/288 (3.1%)</td>
<td>20/288 (7.0%)</td>
<td>-3.9% (92.0%, -5.0%)</td>
</tr>
<tr>
<td>Secondary: Permanent discontinuation</td>
<td>25/288 (8.7%)</td>
<td>50/288 (17.3%)</td>
<td>-8.6% (91.5%, -5.7%)</td>
</tr>
</tbody>
</table>

* Our participant with AEs information pending was excluded from the interim efficacy analysis. * The participant assigned to LAI had treatment discontinuation as the primary endpoint but subsequently experienced VF.
300 Targeting Tat-Dependent Transcriptional Rewiring to Manipulate HIV Latency

William Cisneros, Shimaol Soliman, Miriam Walter, Eun-Young Kim, Ali Shatatfar, Steven M. Wolinsky, Judd F. Hultquist
Northwestern University, Chicago, IL, USA

Background: Integrated HIV-1 proviruses rely on host transcriptional machinery for RNA expression. To enhance transcriptional activity, the virus encodes the transactivating protein Tat, which recruits positive transcription elongation factor b (P-TEFb) to sites of nascent proviral transcription through recognition of the TAR RNA stem loop. P-TEFb phosphorylates the C-terminal tail of DNA polymerase II (RNA Pol II) to enhance processivity and trigger transcriptional elongation. During normal cellular transcription, P-TEFb is recruited to RNA Pol II by the joint action of the PAF1 complex (PAF1C) and the Super Elongation Complex (SEC). Given the apparent functional redundancy between these complexes and Tat, we set out to determine their role in HIV-1 replication and latency.

Methods: HIV-1 spreading infection was monitored following genetic or chemical perturbation of these complexes in primary CD4+ T cells. For genetic perturbation, CRISPR-Cas9 gene editing was used to knock-out each complex member in cells from independent donors. For chemical perturbation, we developed and validated first-in-class small molecule inhibitors of the SEC and PAF1C, which were delivered prior to challenge. These small molecule inhibitors were also tested for their impact on latent proviruses in cell line models of latency and in peripheral blood mononuclear cells (PBMCs) from people living with HIV (PLWH).

Results: Both genetic and chemical perturbation of the SEC and PAF1C significantly increased HIV-1 replication in primary CD4+ T cells, suggesting that these complexes inhibit viral replication. In J-Lat models of latency, neither inhibitor was sufficient for latency reactivation, though they did act synergistically with other latency reversing agents (LRAs). In contrast, both inhibitors significantly increased the expression of HIV-1 gag in PBMCs from PLWH, both individually and with other LRAs. Mechanistic studies suggest that the latency reversing activity of these compounds is Tat-dependent, and that they otherwise promote latency due to an inability to recruit P-TEFb.

Conclusion: These results demonstrate that the SEC and PAF1C inhibit HIV-1 replication and serve as blocks to Tat-dependent transcription in latently infected cells. Small molecule inhibitors of these complexes reactivate latent proviruses in patient PBMCs and act synergistically with other known LRAs. Future directions will explore the Tat-dependency of these compounds and their potential as dual-acting latency reversing and promoting agents.

301 Impact of HIV-1 TAR Sequence Diversity on Its RNA Secondary Structure in HIV-1 DNA Genomes

Mohith Reddy Arikatla1, Pragya Khadka1, Zheng Tang1, Erika Benko1, Colin Kovacs1, Marina Caskey1, Taddeo Kityamweesi2, Paul Boulié3, Stephen Tomuusango4, Aggrey Anok4, Jeffrey Martin5, Melissa Smith1, Timothy J. Wilkin6, R. Brad Jones7, Guinevere Q. Lee1
1Well Cornell Medicine, New York, NY, USA, 2Maple Leaf Medical Clinic, Toronto, Canada, 3The Rockefeller University, New York, NY, USA, 4Kakus Health Sciences Program, Kabuza, Uganda, 5University of California San Francisco, San Francisco, CA, USA, 6University of Louisville, Louisville, KY, USA

Background: HIV-1 TAR is an RNA secondary structure located at the 5’ end of the viral transcript. It binds to HIV-1 Tat protein to facilitate viral transcript elongation and plays a role in virologic rebound during therapy cessation. Here, we hypothesize that TAR secondary structures and stability significantly differ across viral subtypes and across intact versus defective HIV-1 DNA genomes.

Methods: Near-full-length HIV-1 DNA genome sequences from 47 study participants from Uganda (n=28, subtype A1, C, D), Canada and the US (n=19, subtype AE, B) were obtained using nested PCR (HXB2 638-9632) and Illumina sequencing. TAR was inferred from the 3’LTR. Each genome was classified as intact or defective (e.g., containing hypermutations and/or large deletions) using the software HIVSeqInR. To obtain TAR associated with transcription-competent genomes, cell-associated viral mRNA transcripts were sequenced from six matching participants (subtype B n=5, AE n=1) using PacBio IsoSeq. TAR secondary structure and its stability as measured by Gibbs free energy (ΔG) values were inferred using Quikfold; a higher ΔG is associated with decreased TAR stability.

Results: In the HIV-1 DNA genome analysis, among the 47 study donors (1970 TAR sequences), TAR nucleotide diversity associated with intact viral genomes varied up to 13% interhost and 3% intrahost. Subtype D intact genomes had the least stable TAR relative to A and B (p<0.004). TAR in subtype A1 and AE did not share the same secondary structure as subtype B, C and D (linear versus bent hairpins). Per donor, ΔG of TAR associated with intact genomes did not differ from any defective genome categories except was higher when associated with hypermutated genomes (p<0.0006). In the ex vivo viral mRNA transcript analysis, we detected hypermutated genomes that were actively producing viral transcripts which were associated with significantly higher ΔG than non-hypermutated transcripts (p<0.0001), whereas TAR associated with non-hypermutated transcripts had similar ΔG values relative to TARs in the intact viral DNA pool from the same donor (p=0.5).

Conclusion: Our study reveals HIV-1 TAR genotypes are not identical across viral subtypes or between genome-intact versus defective viruses, and they can be genetically diverse intrahost. Differences in TAR stability may imply differences in the extent of viral transcription activity. Future studies should examine whether TAR stability can predict the quantity of viral transcripts produced.

302 Crosstalk Between Resistance to the HIV-1 Capsid Inhibitor Lenacapavir and Viral Fitness

Binh Nguyen, Alex Kleinpeper, Eric O. Freed
National Cancer Institute, Frederick, MD, USA

Background: Lenacapavir (LEN) is the first capsid inhibitor to be FDA-approved for HIV-1 treatment. Despite high potency and slow-release kinetics, a significant drawback of LEN is its low barrier to viral resistance. A mutation in the HIV-1 capsid, M66I, confers >80,000-fold resistance to LEN and has been observed in cultured cells and in HIV-1-infected individuals treated with LEN. However, in the absence of LEN, M66I causes a substantial defect in viral fitness (<5% infectivity relative to WT). Given the high mutation rate of HIV-1, it is important to understand how HIV can adapt to circumvent the M66I-induced fitness defect before compensatory mutations are manifested in patients.

Methods: We propagated LEN-resistant HIV-1 mutants (pNL4-3) in T-cell lines (SupT1 and MT4) to select for compensatory mutations. Replicating viruses were sequenced to identify compensatory mutations. Identified mutations were sub-cloned into pNL4-3 to examine their effects on virus infectivity and drug sensitivity.

Results: M66I propagation in T-cells repeatedly led to WT reversion (I66M). We examined the effects of mutating M66 to other amino acids and determined whether these substitutions recapitulate the behavior of M66I, specifically its resistance to LEN and fitness defect. Of the M66 mutants examined, M66L, M66V, and M66F exhibited similar infectivity defects evident in M66I, but only M66V displayed high-level resistance to LEN. Propagation of these M66 mutations led to several second-site mutations. Of note, H12Y, in combination with A105T and other capsid substitutions, resulted in a >10-fold rescue of M66L infectivity.

Conclusion: This study investigates viral escape strategies of M66I, a highly LEN-resistant but fitness- impaired HIV-1 mutant. As a clinically significant variant, this work will reveal important insights into how HIV-1 may maintain LEN resistance while bypassing the fitness defect inherent to M66I.
Identification of a New Class of Capsid-Targeting Inhibitors That Specifically Block Nuclear Import

Aude Boulay1, Emmanuel Quevarec1, Isabelle Malet1, Giuseppe Nicastro1, Valerie Courgnaut1, Yves L. Janin1, Ian A. Taylor1, Nathalie J. Arbel1

1Univ of Montpellier, Montpellier, France, 2Hôpitaux Universitaires Pitié-Salpêtrière, Paris, France, 3The Francis Crick Institute, London, United Kingdom, 4Institut de Génétique Moléculaire de Montpellier, Montpellier, France, 5Centre National de la Recherche Scientifique, Paris, France

**Background:** Nuclear localized viral genomes account for the persistence of HIV-1 in patients despite successful antiretroviral therapy. Drugs that target reverse transcription or integration do not prevent HIV from reaching the nucleus and producing viral transcripts and proteins. Inhibiting HIV-1 nuclear import could make a significant contribution by reducing the reservoir.

**Methods:** A total of 85 compounds and structural analogues were tested following an in silico screen of compounds that recognize the previously described TRN-1 binding pocket on HIV-1 capsid (Fernandez et al. Nat Microbiol 2019). Antiviral activity was assessed in cell lines and primary human lymphocytes, using X4- and R5-tropic molecular clones, clinical isolates from treatment-naive patients, and ART-resistant mutants. Toxicity and pharmacoKinetic properties were obtained in primary human lymphocytes and humanized mice. Co-immunoprecipitation, surface plasmon resonance, proximity ligation assays, and methyl-Troxy NMR, were used to quantify binding to CA and TRN-1; quantitative PCR to analyse reverse transcripts. 2-LTR circles and integrated provirus; and confocal imaging to measure capsid nuclear import. The effects on uncoating were tested in vitro and in cells, and a total of 16 capsid variants from divergent lentiviruses or stability mutants were compared. Control drugs were NVP, RAL, 3TC, DRV, DTG, PF-74 and LEN.

**Results:** Six compounds (hit H27 and 5 out of 45 structural analogues) inhibited HIV-1 in a dose-dependent manner. The IC50 was in the low micromolar range and CC50 values were >100μM, indicating a high selectivity index. All 6 compounds inhibited the nuclear import of HIV-1 specifically, without impacting other steps of the viral life cycle such as reverse transcription or particle production. Although H27 reduced the TRN-1-CA association, binding sites were distributed over the entirety of CA. All tested HIV-1 molecular clones, clinical isolates, stability or PF74/Len-resistant mutants, and resistant mutants to NRTI, NNRTI, PI and INSTI, were sensitive to H27 (25 viruses in total). In contrast, HIV-2, SIVgag and SIVmac were resistant, and infection with the hypostable P3A8 mutan was even stimulated by H27. Escape mutations to H27 first appeared at day 49 in cell culture and were stability mutants E45L/G46A.

**Conclusion:** This study identifies a new family of capsid-targeting compounds that specifically inhibit HIV-1 nuclear import by modulating the stability of capsid and its ability to bind to nuclear import factors.

Impact of Capsid Polymorphisms on Viral Fitness and the Susceptibility to Lenacapavir

Derek Hansen1, Silvia Chang1, Stephen Yant1, Ross Martin1, Thomas Aeschbacher1, Arthur Cai1, Jason Perry1

1Gilead Sciences, Inc, Foster City, CA, USA

**Background:** Lenacapavir (LEN) is a first-in-class, long-acting capsid (CA) inhibitor for the treatment and prevention of HIV-1 infection. While LEN has shown full potency against different HIV-1 subtypes, it remains unclear how viral diversity encountered in the clinic may impact its efficacy. Herein we analyzed HIV-1 CA sequence diversity to identify natural polymorphisms within the LEN binding site and assessed each for their impact on viral fitness and susceptibility to LEN.

**Methods:** CA binding site residues within a given radius of LEN were identified in Pymol. HIV-1 CA sequences from public (N=9232) and Gilead trial datasets (N=825) were analyzed for naturally occurring binding site polymorphisms across subtypes A, B, C, D, F1, G, CRF01_AE and CRF02_AG. Site-directed mutants encompassing CA polymorphisms with a > 0.5% prevalence were expressed as single-cycle NL4.3-based reporter viruses. Infectivity and antiviral EC50 values for WT and mutant viruses were determined in MT-4 cells.

**Results:** Of the 25 CA amino acids identified within a 5Å radius of LEN at its binding site, 10 (40%) were completely invariant among the >10K unique HIV-1 CA sequences analyzed. Half (5/10) of these conserved residues (M66, Q67, K70, N74 and A105) matched those previously associated with LEN resistance when mutated. Among each of the remaining 15 LEN binding site residues, at least 1 variant was identified across the 8 subtypes evaluated, with codons S41, Q50, T54 and N183 being the most variable with 6, 8, 7 and 9 substitutions detected, respectively. Site-directed HIV-1 reporter viruses encompassing all of the observed CA variants (n=48) were produced and evaluated for infectivity and drug susceptibility in MT-4 cells. Approximately half (25/48) of these mutants showed impaired infectivity (<50%, range 0.006 – 47%) relative to the WT, with 6 (I37Y, Q50P, N53K, T54Y, I73F, R173K) being so severely impaired that it prevented LEN resistance profiling. Of the remaining 42 CA variants, 39 (93%) remained fully susceptible to LEN (FC=0.6 – 2.8). Three variants (Q50E, S56V and S57A), with prevalence of <0.5% in a single subtype (C or D) and impaired infectivity (0.0 – 0.25% of WT), showed reduced susceptibility to LEN relative to WT (FC=3.1, 72 and 4890, respectively).

**Conclusion:** With few exceptions, our mutant HIV panel comprising rare naturally occurring LEN binding site variants in CA remained fully susceptible to LEN, suggesting that the existing natural viral diversity should minimally impact LEN efficacy in the clinic.
Methods: HIV-1env luciferase reporter viruses were pseudotyped with either HIV-1 envelope glycoprotein (Env), murine leukemia virus (MLV) Env, vesicular stomatitis virus G glycoprotein (VSV G), SARS-CoV spike (S) or SARS-CoV-2 S glycoprotein and the effect of producer-cell GBP5 expression on particle infectivity was determined. The effect of GBP5 expression on viral glycoprotein glycosylation was determined by treating virus-producing cell lysates with N-glycosidase F (PNGase F), which removes N-linked oligosaccharides from glycoproteins.

Results: We found that GBP5 reduces the infectivity of particles bearing each of the viral glycoproteins tested in a concentration-dependent manner. Western blot analysis demonstrated that GBP5 causes a dose-dependent shift in the electrophoretic mobility of the viral glycoproteins. Moreover, GBP5 strongly reduced glycoprotein incorporation into virions while increasing virion-associated levels of the uncleaved glycoprotein precursors. PNGase F treatment abolished the GBP5-mediated shift in the electrophoretic mobility of the glycoproteins, indicating that GBP5 affects N-linked protein glycosylation and glycan modification.

Conclusion: Our data establish that GBP5 impairs viral infectivity by interfering with glycoprotein function. Furthermore, we provide evidence that GBP5 not only inhibits furin cleavage of viral glycoproteins but also affects their glycosylation regardless of whether they undergo furin-dependent processing. Moreover, our data on VSV G indicate that GBP5 targets the glycosylation of proteins other than class I fusion proteins. These results provide novel insights into the broad antagonism of viral glycoprotein function by the cellular host innate immune response. In the ongoing studies, we are testing the ability of GBP5 to restrict the replication of human and murine pathogens in mouse models, thereby providing the first insights into GBP5 antiviral activity in vivo.

307 Retinoic Acid Blunts the Aryl Hydrocarbon Receptor/SAMHD1-Dependent HIV-1 Restriction in Macrophages

Ramon Edwin Caballero 1, Jonathan Diaz 1, Jean-Philippe Goulet 1, Jean-Pierre Routy 1, Andrew Moulard 1, Petronela Ancuta 1, 1Centre de Recherche du CHUM, Montreal, Canada, 2Collège, Montreal, Canada, 3McGill University Health Centre Research Institute, Montreal, Canada, 4McGill University, Montreal, Canada

Background: As part of the cell-autonomous innate immune system, SAMHD1 restricts HIV-1 replication by limiting the pool of deoxyribonucleoside triphosphate required for efficient reverse transcription. The antiviral activity of SAMHD1 is abrogated by phosphorylation. Among regulators of SAMHD1 activity, ligands of aryl hydrocarbon receptor (AhR) were reported to decrease SAMHD1 phosphorylation via mechanisms involving the transcriptional repression of kinases CDK1/2. Conversely, we demonstrated that retinoic acid (RA) promotes HIV-1 replication in macrophages by inducing SAMHD1 phosphorylation. Considering the role of macrophages in HIV-1 infection in anatomic sites rich in both AhR ligands and RA, such as the intestine, here we investigated how the crosstalk between these two pathways governs SAMHD1 activity and HIV-1 replication in macrophages.

Methods: Monocyte-derived macrophages (MDM) generated in the presence of M-CSF were exposed to all trans RA (ATRA) and/or AhR agonist (FIIC2) and exposed to replication-competent or single-round VSV-G- pseudotyped HIV-1. HIV replication was measured by ELISA, FACs, and nested real-time PCR using specific primers for early/late reverse transcripts and integrated HIV-DNA. RNA-Sequencing was performed using the Illumina technology. Validation were performed by Western Blot, RT-PCR or FACs.

Results: While exposure to FIIC2 inhibited HIV-1 replication in MDM at the level of reverse transcription, integration, and translation, this antiviral effect was absent in the presence of ATRA, which significantly increased HIV-1 replication via CCR5-dependent entry and post-entry mechanisms. FIIC2 decreased SAMHD1 phosphorylation in the absence but not in the presence of ATRA, consistent with the robust RA-mediated increase in CDK1 expression. Similarly, the insulin-induced gene 1 (INSIG1), involved in Gag metabolism, was induced by FIIC2 only in the absence of ATRA. The analysis of differentially expressed genes in ATRA-treated MDM revealed the downregulation of the AhR nuclear translocator (ARNT) and other AhR target genes, and the upregulation of the gene for AhR repressor (AhRR), indicating a blunted AhR signaling pathway.

Conclusion: Our results demonstrate opposing roles for AhR and RA pathways in the modulation of the antiviral activity of SAMHD1 and reveal a blunted AhR-mediated antiviral program in MDMs in the presence of RA. These studies identify the need for natural SAMHD1 modulators as novel therapeutic targets for HIV-1.

308 Monitoring the Infection of a New Strain of Simian Betaretrovirus in Southern Murineis from Brazil

Thamiris S. Miranda 1, Pedro H. Carneiro 2, Marcus Vinicius de Mattos Silva 1, Raoldado da Silva Mohana Borges 1, Silvia B. Moreira 1, Aicles Pisinvesti 1, Orlando da Costa Ferreira Junior 1, Marcelo A. Soares 1, André Felipe Andrade dos Santos 1
1Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 2Centro de Primatologia do Rio de Janeiro, Rio de Janeiro, Brazil

Background: Neotropical primates are natural hosts of several viruses, but many are poorly studied. One example was the identification through massive sequencing of a new strain of simian betaretrovirus (SRVbar) in an individual (2506) of Southern Murineis (Brachytwes arnachoides), a critically endangered species, who died of immunodeficiency at the Primatology Center of Rio de Janeiro (PRJ). The phylogenetic analysis showed that the SRVbar group close to the SRV of Asian primates, which also causes clinical signs similar to those presented by Muriqui. Four muriquis from the same enclosure as 2506 were also positive for SRVbar, but were asymptomatic. Objective: The objective of the study was to monitor the infection of SRVbar in specimens 2506 and asymptomatic contacts.

Methods: Oral swab and blood samples were collected from five muriquis in CPRJ between 2016 and 2020. Nucleic acids were extracted from plasma, peripheral blood mononuclear cells (PBMC) and oral swab and a qPCR was performed for the env to analyze the proviral and viral loads from SRVbar. For serological analysis, a recombinant SRVbar p27Gag protein produced on the Escherichia coli cell platform was synthesized and purified by affinity chromatography to be used as a viral antigen in the Western Blot.

Results: The proviral load in the PBMC was 10x higher than in the oral swab of all animals tested (p=0.0001). Specimen 2506 showed similar proviral DNA loads in PBMC and oral swab 10x higher (log 8.12/10⁶ copies/10⁶ cells, respectively) than compared to asymptomatic animals (log 7.66 copies/10⁶ cells and 6.57 copies/10⁶ cells, respectively) (p=0.038). All asymptomatic animals had detectable viral loads and the average was 2.34 log₉ copies/mL of plasma (1.4 - 3.18, min-max) while 2506 had a viral load greater than 3.96 log₉ copies/mL of plasma (p=0.044). There was success in the production of the recombinant SRVbar p27Gag protein and seropositivity for this antigen varied between animals over time, where for two animals (2506 and 3078) there was an inverse relationship with viral load, as already described in Asian primates of the genus Maraca infected with SRV.

Conclusion: For the first time, a study on the course of betaretrovirus infection being investigated in Neotropical primates, contributing to the knowledge of this new viral strain.

309 WITHDRAWN

310 Species-Specific APOBEC3 Splicing and Variations in Viral Mutagenesis

Azad Khoob 1, Rachael Springman-Rodriguez 2, Armando Mendez 1, Kathryn Jackson-Jones 1, Margarita Zhetskaya 1, Judd F. Hultquist 1, Diako Ebrahimi 1
1University of Texas at San Antonio, San Antonio, TX, USA, 2Northwestern University, Chicago, IL, USA

Background: APOBEC3 (A3) proteins are known for their antiviral activities, acting as restriction factors to directly mutate viral genomes or block different stages of viral replication. Among the seven A3 enzymes in humans, A3G is the most restrictive enzyme, unique in its ability to cause G to A mutations that can generate stop codons. While HIV-1 Vif counteracts A3 enzymes by targeting them for ubiquitination and proteasomal degradation, G to A mutations are still common in clinical isolates, and sublethal levels of A3-induced mutations them for ubiquitination and proteasomal degradation, G to A mutations are still common in clinical isolates, and sublethal levels of A3-induced mutations are being investigated in Neotropical primates, contributing to the knowledge of this new viral strain.

Results: Our investigation revealed that this splicing defect is present in many Old-World monkeys but not in Great Apes and New-World monkeys, and that the mutational signatures of SIV reflect this immune defect. Contrary to the
previous report, we found that A3G mRNA splicing defect is independent of the insertion of an Alu element in the A3G locus. Instead, it is strongly associated with a small intronic variation in only rhesus macaques and other similar nonhuman primates used frequently in HIV studies.

**Conclusion:** Taken together, our studies have unveiled and characterized a significant difference in A3G-mediated anti-HIV/SIV immunity across human and nonhuman primate models. These findings have significant implications for our understanding of the disparities in viral resistance, drug resistance and immune evasion mechanisms between humans and nonhuman primate models of HIV.

### 311 High Replication Fitness and Potent Innate Immune Evasion Function of a HIV-1 VB Strain

**Dorota J. Kmiec,** Kerstin Regensburger, Frank Kirchhoff

**University Medical Center, Ulm, Germany**

**Background:** A highly virulent subtype B (VB) HIV-1 has been detected in >100 individuals (Wyant et al., 2022). On average, infected patients show 5-fold higher viral loads and 2-fold faster CD4+ cell loss compared to other subtype-B infected individuals. To determine molecular determinants of its virulence, we functionally characterized a clone (VB-6) of this HIV-1 variant.

**Methods:** HIV-1 VB-6 isolates were found to be most similar to VB consensus and used to synthesize an infectious molecular clone. Following transfection into HEK293T cells to generate infectious virus, particle infectivity, cold stability and ability to infect primary T cells and monocyte-derived macrophages were examined. Replication kinetics of VB-6 in activated T cells were compared to 8 other HIV-1 subtype B strains. Sensitivity to CCR5 and CXCR4 inhibitors was measured in primary T cells by flow cytometry. Resistance to IFI16 and activation of NF-κB reporter were tested by co-transfection of the molecular clone into HEK293T cells. Induction of cell death, modulation of immune receptors and proinflammatory cytokine release by VB-6 were determined by flow cytometry.

**Results:** VB-6 had average particle infectivity and stability, and like most primary subtype B strains is F-cell tropic and utilizes CCR5 entry co-receptor. However, the replication fitness of VB-6 in T cells was superior to all tested (8) primary subtype B CCR5-tropic strains, including 3 transmitted-founder viruses. VB-6 potently downmodulated immune receptors (CD4, CCR5, CXCR4 and tetherin) from the cell surface using Vpu and Nef in a more efficient manner than the NL4-3 reference strain. VB-6 infection of primary T lymphocytes induced increased levels of cell death and proinflammatory cytokines, while suppressing type I and III IFN production. In addition, VB-6 was more active at inducing NF-κB signaling and more resistant to IFI16 than most other tested subtype B strains.

**Conclusion:** The hyper-virulent HIV-1 VB-6 strain shows high replicative fitness in primary human lymphocytes and expresses Vpu and Nef proteins that potently downmodulate immune receptors. VB-6 is also adept at downmodulating tetherin and reduces sensitivity to inhibition by IFI16. In line with increased replication, VB-6 infected primary T cells produce more proinflammatory cytokines and show reduced viability. A combination of potent immune evasion and high replicative fitness likely contribute to the virulence of VB-1 HIV.

### 312 The HIV-1 Protein ASP Promotes Viral Replication and Is Associated With Faster Disease Progression

Mohd Shameel Iqbal1, Myriam Houmey2, Ziyan Xu2, Yuchu Wang2, Kabir Khan2, Isabella Caico2, Rui Li2, Jean-Michel Mesnard3, Nathalie Chazal3, Fabio Romero1

1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2University of Montpellier, Montpellier, France, 3University of Padova, Padova, Italy

**Background:** Decades after its discovery, the HIV-1 antisense gene asp remains an enigma. Asp overlaps env and is present exclusively in pandemic group M HIV-1 strains. Thus, its creation may have improved HIV-1 fitness or spread. We reported that ASP is found on the surface of HIV-1 virions. We studied how ASP expression impacts HIV-1 entry and replication and the underlying mechanisms. We also studied the evolutionary mechanisms that created asp, and its association with disease progression.

**Methods:** We used fusion and infection assays to assess how ASP expression impacts HIV-1 entry and replication. Identification and molecular validation of ASP interacting partners were performed by mass spectrometry (MS) and co-immunoprecipitation (Co-IP). Functional validation of MS results was carried out by studying ASP nuclear import and its impact on DNA repair, as well as the impact of ASP on mitochondrial function. We used computational methods to identify the evolutionary mechanisms that created the asp gene and its association with HIV-1 disease progression.

**Results:** We created asp deficient HIV-1 strains by mutating the start codon or by introducing early stops via single nucleotide substitutions that lead to synonymous codons in env. ASP-deficient strains show reduced fusion and replication capacity compared to wildtype. To determine potential mechanisms, we identified ASP binding partners by MS and validated the results by co-IP. We found that the Transportin 1 pathway mediates ASP nuclear import, and that in the nucleus ASP interacts with factors involved in multiple DNA damage repair pathways. Functional studies confirmed that ASP promotes DNA repair. Also, ASP interacts with and increases the expression of mitochondrial factors of the oxidative phosphorylation and ATP biosynthesis pathway. Finally, sequence analyses show that creation of asp occurred via evolution of codon usage in env involving differential use of synonymous codons or conservative amino acid substitutions that eliminated early stops in asp. While presence of asp constrains env genetic diversity, it provides a selective advantage as the presence of asp is significantly more frequent in rapid progressors than long-term nonprogressors.

**Conclusion:** For the first time our studies link a full-length asp ORF to HIV-1 entry and replication in vitro and disease progression in vivo. We identified possible molecular mechanisms including impact of ASP on DNA repair and mitochondrial function, and the evolutionary mechanisms that created asp.

### 313 In Vivo Detection of HIV-1 Antisense Transcripts in Donors Before and During ART

Adam A. Caporferi1, 2, Tolleke O. Famuyina3, 1, Rachel Sklutuski3, 1, Sacha Pathak1,Jennifer L. Groebner1, Rui Li2, Jason W. Rausch3, Steven G. Deeks3, John W. Mellors4, John M. Coffin5, Fabio Romero1, Mary F. Kearney3

1National Cancer Institute, Frederick, MD, USA, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 3University of California San Francisco, San Francisco, CA, USA, 4University of Pittsburgh, Pittsburgh, PA, USA, 5Tufts University, Boston, MA, USA

**Background:** Natural antisense transcripts (NATs) are expressed by viruses, prokaryotes, and eukaryotes and primarily function in regulating sense gene expression through multiple mechanisms. In vivo studies have shown that HIV-1 expresses antisense transcripts (AST) from a Tat-independent negative sense promoter in the 3'LTR. In cell line studies, AST was demonstrated to promote HIV latency through epigenetic modification of histones in the 5'LTR by the Polycymerase Complex 2 (PRC2). Here, we asked whether HIV AST is expressed in infected PBMCs collected from untreated and ART-treated donors.

**Methods:** PBMCs were obtained from 10 donors in the SCOPE cohort and 2 donors from University of Pittsburgh. Untreated donors were either ART-naïve or undergoing treatment interruption with viral loads ranging from <50–200,000 copies/ml of plasma (n=10). Donors on ART had viremia suppressed (<50 copies/ml) for a median of 5.4-years (n=3). AST levels were measured by cell-associated antisense RNA single-genome sequencing (SGS) of a 1.7kb fragment in the opposite orientation of the env coding region. An endpoint digital PCR approach with tagged-cDNA and donor-specific primers was also used to quantify AST copies in the samples.

**Results:** We detected HIV AST in 11/12 donors with a median of 14 [IQR 5–34] copies/100 infected PBMCs (Figure-Left Panel). Antisense SGS revealed that about 5% of infected PBMCs collected from donors on ART contained AST at any given time. The genetic diversity of the antisense transcripts was consistent with expression from a diverse population of proviruses. Proximal clonal populations were identified with matched HIV AST from multiple aliquots of single-infected PBMC (Figure-Right Panel). Digital PCR showed similar levels of AST expression in untreated donors with varying levels of plasma viremia. Further, in the donors on ART, we observed no statistical difference between the levels of sense and antisense transcripts.

**Conclusion:** As in the case of other NATs, HIV antisense transcripts are expressed at low levels in both ART-treated and untreated individuals. The in vivo expression of AST irrespective of treatment status warrants further investigation into its potential role as a long non-coding RNA capable of regulating HIV-1 sense gene expression and inducing HIV latency. Understanding the role of HIV AST in vivo may inform future strategies for controlling HIV replication without ART.
Exacerbation of the RSV Infectivity by SARS-CoV-2 in an In-Vitro Coinfection Cellular Model

Claudia Vanetti, Silvia Zacchini, Gioia Cappelletti, Micaela Garziano, Irma Saulle, Sergio Strizzi, Fiona Limanagi, Claudio Fenizia, Claudia Moscheni, Antonella Tosoni, Manuela Nebuloni, Mario Clerici, Daria Trabattoni, Mara Biasin

University of Milan, Milan, Italy

Background: Concurrent infections with two or more pathogens with an analogous tropism, such as Respiratory Syncytial Virus (RSV) and SARS-CoV-2, may antagonize or facilitate each other modulating host disease outcomes. Clinically, a severe phenotype has been reported in children with RSV/SARS-CoV-2 co-infection. However, experimental models to study the cellular, molecular and immunological dynamics of co-infections are extremely limited. Herein, we propose an in-vitro co-infection model to assess RSV/SARS-CoV-2 immune and viral evolution.

Methods: A549-ACE2 expressing cells were single or co-infected with RSV and SARS-CoV-2 (MOI=0.01 each) (Figure 1A). SARS-CoV-2 and RSV replication was assessed at 24, 48 and 72 hours post infection (hpi) by Droplet Digital PCR (ddPCR), immune-fluorescent (IF) and transmission electron microscopy (TEM) analyses. Secretome analyses (17 Multiplex Cytokine ELISA) on cell culture supernatants and anti-viral/immune/autophagy gene expression (RT-qPCR) were evaluated as well. All the experiments were performed in the BSL3 facility.

Results: The RSV/SARS-CoV-2 co-infection was characterized by a significant increase in the replication rate of RSV (co-infection vs single infection p<0.001) (Figure 1B). The co-infection was able to modulate the viral host receptors' expression, as significant increase in ICAM1 expression, one of the RSV host receptors, was observed in the co-infected condition compared to the uninfected control (p<0.0001) and to the RSV (p<0.0001) and SARS-CoV-2 (p<0.0001) single infections. Remarkably, co-infection was accompanied by a significant rise in the expression of pro-inflammatory genes, further confirmed by secretome analysis. Moreover, substantial morphological changes were evident in the co-infected A549-ACE2 cells showing an increase in the number and length of cellular conduits. Finally, following co-infection, cells displayed evident in the co-infected A549-ACE2 cells showing an increase in the number and length of cellular conduits. Finally, following co-infection, cells displayed a significant increase in LC3B gene expression (p<0.05), which was further confirmed by IF analysis, suggestive of an alteration of the autophagy pathway.

Conclusion: The RSV/SARS-CoV-2 co-infection model displays a unique and specific viral and molecular fingerprint. These findings give clues of augmented severity upon RSV infection in the context of a concomitant SARS-CoV-2 co-infection. This in-vitro co-infection model may represent an attractive, cost-effective approach to mimic both viral dynamics and host immune responses, providing readily-measurable targets predictive of co-infection progression.
characterize unconventional T cell phenotypes in 25 HIV-exposed seroconverted (HESC) individuals prior to seroconversion and compare them to HIV-exposed seronegative (HESN) individuals.

**Methods:** RV227 is a prospective natural-history study which recruited individuals at high risk for HIV acquisition. PBMC from the RV227 cohort were used to investigate T cell phenotypes in 25 HESC individuals prior to seroconversion. Each HESC study participant was matched for age, gender, and risk behavior with 3 HESN individuals for comparative analysis. A multicolor flow cytometry panel was developed to investigate mucosal-associated invariant T (MAIT), invariant natural killer T (iNKT), gamma delta T, and CD4+ T cell phenotypes prior to HIV seroconversion.

**Results:** Our analysis showed increased frequency of total α4β7 expressing CD4+ memory T cells in HESC individuals (%α4β7+, p=0.075; %α4β7+CD4+, p=0.0030) compared to HESN individuals. Additionally, our study shows increased frequency of α4β7 expressing iNKT cells in HESC individuals compared to HESN individuals (%α4β7+, p=0.0039). To further characterize α4β7 expressing iNKT cells, we analyzed α4β7 expression in major iNKT cell subsets: CD4+ iNKT cells, CD8+ iNKT cells and CD4+CD8- (DN) iNKT cells. Our results reveal that α4β7 expression in HESC individuals is increased in CD4+ iNKT cells (p=0.0342) and not CD8+ iNKT cells (p=0.5424) or DN iNKT cells (p=0.7334) compared to HESN individuals. No differences were found in MAIT and gamma delta T cells.

**Conclusion:** Our study supports the existing role of α4β7+/α4β7− CD4+ memory T cells in HIV susceptibility and identifies a potential role for gut homing α4β7+/α4β7− iNKT cells in HIV susceptibility in the context of individuals at high risk for HIV acquisition.

### 317 Mobility of Transmitted/Founder HIV-1 Variants in Human Cervicovaginal Mucus

**Matrona M. Akiso**, Marianne Mureithi, Sarah Joseph, Ann M. Carias, Omu Anzala, Thomas Hope

**University of Nairobi, Nairobi, Kenya, Imperial College London, London, UK, Northwestern University, Chicago, IL, USA**

**Background:** HIV-1 needs to traverse the mucus barrier overlaying the mucosal epithelia for infection of the target cells to occur. A single or a limited number of HIV-1 variant(s), known as Transmitted/Founder (T/F) HIV-1, are typically responsible for successive infections, overcoming the mucosal barriers, including the protective mucous layer. Factors affecting cervicovaginal mucus physical and chemical properties may influence its barrier function. How this influences the mobility of T/F HIV-1 variants in the mucus is yet to be determined. Our study sought to unravel how factors affecting the cervicovaginal mucus properties, such as serum levels of reproductive hormones, cervicovaginal pH, microbiome, immunoglobulin content and cytokines, may influence the mobility of T/F HIV-1 variants in cervicovaginal mucus.

**Methods:** A cross-sectional evaluation was conducted to assess the mobility of three T/F HIV-1 clades in cervicovaginal mucus from at least 80 adult women, both HIV-infected and uninfected, aged between 18 and 45 years in Nairobi, Kenya. Spearmen correlation was used to correlate the mobility data to the cervicovaginal mucus pH, serum estradiol and progesterone levels and age. A t-test and ANOVA were used for comparing viral mobility between different HIV-1 infection statuses, bacterial vaginosis statuses, and viral clades, respectively.

**Results:** Our results show a significant impediment to the mobility of T/F HIV-1 variants in the cervicovaginal mucus. We observed significant variabilities in the mobility of the different T/F HIV-1 clades in the cervicovaginal mucus. Clade B lab adapted strain (R9 891) and T/F HIV-1 variant (CH040) had the lowest mobility compared to clade C (CAP045) and the CRF_AE (92TH023). The mobility of the CRF_AE (92TH023) negatively correlated to the mucus pH and viscosity. Interestingly, we observed significant positive mobility correlations among some viral clades.

**Conclusion:** Our study results demonstrate that cervicovaginal mucus impedes the mobility of T/F HIV-1 variants, which varies among clades. These results also show that cervicovaginal microenvironment may influence the mucus barrier function. This study is crucial for gaining insights into the biological traits of T/F HIV-1 variants, enabling them to bypass the mucosal immune bottleneck. By understanding these early interaction events, we can facilitate the development of preventive strategies and treatments that enhance mucosal barrier function, thus reducing the transmission of HIV-1.

### 318 IL7RA rs10491434 Polymorphism Is Related to Spontaneous HIV Infection Control: A Retrospective Study

Daniel Sepulveda-Crespo, Maria A. Jimenez-Sousa, Amanda Fernandez-Rodriguez, Maria A. Munoz-Fernandez, Jose L. Jimenez, Jorge del Romero, Sergio Reus Bañuls, Helem Vilchez, Beatriz Motte, Ixobor Martinez, Jose M. Benito, Norma Raillon, Salvador Resino

**Institute of Health Carlos III, Madrid, Spain, University Hospital Gregorio Maranon, Madrid, Spain, Centro Sandeolano, Madrid, Spain, Hospital General Universitario de Alicante, Alicante, Spain, Hospital Universitario de los Ezpeletas, Palma de Mallorca, Spain, IrsiCaixa Institute for AIDS Research, Badalon, Spain, Instituto de Investigacion Sanitaria Fundacion Jimenez Diaz, Madrid, Spain**

**Background:** IL7 receptor (IL7R) is vital in the adaptive immune response against HIV. We assessed IL7RA polymorphisms (SNPs) in antiretroviral therapy (ART)-naïve HIV patients for their association with spontaneous HIV infection control.

**Methods:** We conducted a retrospective cohort study involving 667 ART-naive patients categorized by HIV progression (ordinal variable): 150 rapid progressors, 334 moderate/typical progressors, 86 long-term nonprogressors. The number of elite controllers (LTNs-EC), and 97 LTNPs-non-EC. We genotyped three IL7RA SNPs using Agena Biosciences’ MassARRAY platform. The association between IL7RA SNPs and spontaneous HIV infection control was evaluated using ordinal logistic regression.

**Results:** Individuals carrying the rs10491434 G allele have a higher likelihood of spontaneous HIV infection control (adjusted odds ratio (aOR)=1.13; p=0.023). Moreover, the IL7RA GCT haplotype, consisting of three specific SNPs (rs6897932, rs987106, and rs10491434), demonstrated an association with the control of untreated HIV infection (aOR=1.34; p=0.050). Remarkably, the rs10491434 SNP and the IL7RA GCT haplotype exhibited similar aOR values, suggesting that rs10491434 may be primarily responsible for the observed effect of the haplotype.

**Conclusion:** IL7RA rs10491434 G allele is associated with a higher likelihood of spontaneous HIV infection control, indicating its significant role in the pathogenesis of HIV, possibly influencing infection course and viral replication control.

### 319 Genetic Variants Associated to HIV Control Are Associated With NK Cell Markers and Response to CMV


**Radboud University Medical Center, Nijmegen, Netherlands, Erasmus University Medical Center, Rotterdam, Netherlands**

**Background:** Genome-wide association studies (GWAS) have shown that genetic variants in the MHC region are associated with spontaneous HIV-1 control. How these variants affect immune functioning is, however, poorly understood. We used a functional genomics approach in which we integrated GWAS with transcriptomic, plasma proteomic and functional immunological data to understand how genetics contribute to HIV control.

**Methods:** 1380 people living with HIV of European ancestry were included as part of the 2000HIV study (clinicaltrials.gov NCT03994835). To find genetic variants associated to control, a GWAS was performed in 67 HIV controllers (HIC) and 272 matched non-HIV controllers on ART (non-HIC) using a logistic regression model. To unravel how genetics affects phenotype, we performed quantitative trait locus (QTL) mapping with data from the 2000HIV cohort. A linear regression model was used to associate genetic dosages with: 1) cytokine production upon ex vivo PBMC stimulation with various stimuli (QTLs), 2) levels of 2367 plasma proteins as measured by Olink technology (pQTLs), 3) bulk RNA expression in PBMCs (eQTLs) measured by RNA-sequencing in 1146, 1222 and 1208 individuals, respectively. All genome-wide significant (P < 5 × 10−8) QTLs were intersected with suggestive GWAS loci.

**Results:** In agreement with previous GWAS, the strongest association with HIV control was identified in the MHC locus (Fig A), where we found 8 independent QTLs associated to control, a GWAS was performed in 67 HIV controllers (HIC) and 272 matched non-HIV controllers on ART (non-HIC) using a logistic regression model. To unravel how genetics affects phenotype, we performed quantitative trait locus (QTL) mapping with data from the 2000HIV cohort. A linear regression model was used to associate genetic dosages with: 1) cytokine production upon ex vivo PBMC stimulation with various stimuli (QTLs), 2) levels of 2367 plasma proteins as measured by Olink technology (pQTLs), 3) bulk RNA expression in PBMCs (eQTLs) measured by RNA-sequencing in 1146, 1222 and 1208 individuals, respectively. All genome-wide significant (P < 5 × 10−8) QTLs were intersected with suggestive GWAS loci.
Conclusion: We showed that genetic variants associated to HIV control are associated to plasma and gene expression levels of NK cell receptors, their ligands MICA/MICB and HLA-C, and effector molecule granzyme A. This suggests that these variants might contribute to HIV control by modulating NK cell function. The association of variants in the MHC locus with immune responses against CMV hints to a shared genetic basis of immune responses against HIV and CMV. Finally, we showed that our QTL mapping studies are valuable resources that can aid understanding how genetics contributes to disease outcome in PLWH.

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320 Plasma Proteome Signature of Immune Inhibition Is Associated With CMV Coinfection in People With HIV
Michael L. Freeman1, Gordon Honkamp Smith1, Patricia K. Riggins1, Scott L. Letendre2, Peter W. Hunt3, Sara Ganella Weibel1
1Gree Western Reserve University, Cleveland, OH, USA, 2University of California San Diego, La Jolla, CA, USA, 3University of California San Francisco, San Francisco, CA, USA

Background: Cytomegalovirus (CMV) coinfection is highly prevalent and is associated with persistent inflammation in people with HIV (PWH). A comprehensive analysis of the plasma proteome in PWH with or without CMV has never been performed previously. Identifying proteomic signatures of CMV infection in PWH will improve understanding of CMV-associated comorbidities in PWH and may lead to the development of novel therapeutics.

Methods: The Olink Proteomics proximity extension assay was used to characterize the plasma proteome from age- and CD4 count-matched PWH with (CMV+, n=30, 23.3% female) or without CMV (CMV-, n=19, 10.5% female) CMV coinfection on suppressive antiretroviral therapy (ART). Resulting normalized protein expression (NPX) values were analyzed using a linear mixed effects model to determine protein expression differences between groups. P-values were adjusted using the Benjamini-Hochberg method. Partial least squares-discriminant analysis (PLS-DA) was used to discriminate between CMV groups using the NPX values as input features. To measure the predictive power of the PLS-DA model, we used five-fold cross-validation with 100 repeats to estimate the generalization error in terms of accuracy and area under the receiver operating characteristic (ROC) curve (AUC).

Results: We found that 117 of 368 (31.8%) proteins were differentially expressed (P<0.05) after adjustment for multiple comparisons. Proteins most significantly upregulated in CMV+ participants were associated with inhibition of the immune response (e.g., FCRL6, HLA-DRA, KLRD1, CD70, LY9, SLAMF7) or inflammation (e.g., CXCL9, CXCL10). Several of the most significantly downregulated proteins in CMV+ PWH were associated with apoptosis (e.g., CASP2, TRIM21, TRAF2). Using our PLS-DA model, we were able to predict CMV serostatus with a cross-validated accuracy of 80.8% and AUC of 87.8%.

Conclusion: CMV serostatus substantially influences protein expression with nearly a third of proteins differentially expressed. Our data demonstrate that CMV is a major contributor to the plasma proteome in PWH and suggest that targeting CMV in PWH may be a viable strategy for therapeutic interventions. While the cross-sectional design limits causal inference, the increase in immune inhibitory elements and the reduction of pro-apoptotic molecules in CMV+ PWH suggest that CMV may facilitate the maintenance of the latent HIV reservoir in PWH on suppressive ART.

321 B-Cells Augment HIV-1 Expression in Secondary Lymphoid Tissues
Matthew Oliver1, Joy Folkvord1, Cecilia Shikuma2, Scott Yost2, Magali Porouchia1, Davey M. Smith1, Sara Ganella Weibel1, Elizabeth Connick1
1University of Arizona, Tucson, AZ, USA, 2University of Hawaii, Honolulu, HI, USA

Background: HIV-expressing (vRNA+) cells are consistently found in secondary lymphoid tissues (SLT) of people with HIV (PWH) on antiretroviral therapy (ART) and likely drive viral rebound upon ART cessation. Little is known about what affects HIV expression in SLT. We hypothesized that B cells promote HIV expression.

Methods: Disaggregated tonsil cells from persons without HIV were depleted of CD19+ cells by magnetic beads. CD19- cells were spincoated with X4 or R5-tropic HIV GFP reporter virus, cultured 3 days with dye-labeled CD19+ or CD19- cells and saquinavir, and %GFP+ of unlabeled CD3+ CD8- cells and GFP median fluorescence intensity (MFI) determined by flow cytometry. Tonsil cells were also sorted into CD4+ T follicular helper (TFH; CD3+ CD8- CXCR5hiPD-1hi), nonTFH (CD1+ CD8-CXCR5+/PD-1+/+), memory B (IgD-CD38-), pre-germinal center B (pre-GCB; IgD+CD38+), GC B (IgD+CD38+), and naive B (IgD+CD38-). TFH and nonTFH were infected with X4 HIV, incubated with B cell subsets or control CD4+ T cells, and %GFP+ and GFP MFI determined. Sections of inguinal lymph node (LN) from 6 male PWH on ART for 7-29 years and spleen from 6 male PWH on ART for >5 years were analyzed by in situ hybridization for vRNA and immunofluorescent antibody staining for CD20 to determine follicular (F) regions. Frequencies of vRNA+ cells in F and extrafollicular (EF) regions, EF B cells, and EF B cells adjacent to vRNA+ cells were determined by visual inspection and quantitative image analysis. Data reported are medians. Nonparametric statistical tests were used.

Results: CD19 depletion reduced %GFP+ and GFP MFI by 32% and 30% in X4 (n=7; p=0.02 and 0.03) and 3% and 29% in R5-HIV infected cells (n=7; both p=0.02) compared to cultures with CD19+ cells. Memory, pre-GCB, and to a lesser extent naive B cells elevated HIV expression in both nonTFH (%GFP+ p<0.05; GFP MFI p<0.05; n=6) and TFH (%GFP+ increased by all B cell subsets except naive, p<0.05; n=6); all B cell subsets augmented GFP MFI (p<0.05; n=6). Frequencies of vRNA+ cells were higher in B cell-rich F than EF regions in LN (0.28 vs 0.17 cells/mm²; p=0.03) and spleen (0.07 vs 0.05 cells/mm²; p=ns). In EF areas, vRNA+ cells were adjacent to B cells more often than predicted by chance in all four LN (3.1-fold; range, 1.5-2.4) and four spleens (1.4-fold, range 1.1-2.1).

Conclusion: B cells upregulate HIV expression in SLT CD4+ T cells and are associated with vRNA+ cells in SLT of PWH on ART. Mechanisms by which B cells augment HIV expression merit investigation.

322 Herpesviruses Reactivation and Associated Systemic Inflammation During Initiating ART, Rakai-Uganda
Victor Ssempijja1, Viviane Callier1, Martha Nason1, Aggrey Anok2, Andrea Lisco3, Andrew Redd4, Thomas C. Quinn1, Adam Rupert4, Stephen Tomusange4, Taddjo Kityamwensu4, Paul Bhuule5, Irini Sereti6, Steven J. Reynolds7, Stephen Tomusange8, Andrew Redd9, Andrew Redd9
1Julius Biomedical Research, Inc, Frederick, MD, USA, 2National Institutes of Health, Bethesda, MD, USA, 3National Institutes of Health, Bethesda, MD, USA, 4Rakai Health Sciences Program, Kalisizo, Uganda, 5National Institutes of Health, Bethesda, MD, USA

Background: We conducted a prospective observational study to evaluate HSV-2, Cytomegalovirus (CMV), and HHV-8 herpesviruses shedding after ART initiation and associated markers of systemic inflammation in women living with HIV (WLWH).

Methods: We recruited 187 WLWH not on ART, aged ≥18 years from three HIV/ART clinics in south-central Uganda. CMV and HSV-2 shedding was quantified by PCR on vaginal secretions and HHV-8’s shedding on oral swabs. McElmaw’s test was used to evaluate changes in shedding of HSV-2, CMV and HHV-8 pre-ART (study baseline visit) and after ART initiation (weeks 4 and 8). Logistic regression models were used to evaluate associations of markers of systemic inflammation (plasma levels of IL-6, CRP, TNFα or sCD14) with viral-shedding at similar time points. We conducted a stratified analysis by pre-ART CD4 counts (≤200 cells/µl versus >200 cells/µl).

Results: 187 out of 196 (95%) screened women were eligible with a mean age 30.1 years and enrolled at ART initiation. 25.9% of participants had a pre-ART CD4 count less than 200 cells/µl. Follow-up occurred for 91% and 87% of participants at weeks 4 and 8 post-ART initiation, respectively. During the study, 65.1% of participants shed CMV, 44.1% shed HSV-2, and 30.1% shed HHV-8. Shedding of HSV-2 and HHV-8 pre-ART versus at week-4 or week-8 post-ART was not significantly different while CMV shedding significantly increased from 53% pre-ART to 77% at week-4 visit (p-value = 0.016), and 73% at week-8 visit (p-value = 0.027) in participants with a pre-ART CD4 count <≤200 cells/µl. At week-4 post-ART, a 4-fold increase in IL-6 levels was associated with HSV-2 shedding if there was no shedding pre-ART, while a 0.76-fold decrease in IL-6 levels was associated with HSV-2 shedding if there was shedding pre-ART. At week 8 post-ART, a 2-fold and 8-fold increase in CRP and TNFα levels respectively...
were associated with the risk of CMV shedding irrespective of shedding pre-ART. A 4-fold and 1.5-fold increase in IL-6 and sCD14 levels respectively was instead associated with HSV-2 shedding if there was no shedding pre-ART while at least a 0.17-fold decrease in IL-6 and sCD14 levels was associated with HSV-2 shedding if there was shedding at baseline.

**Conclusion:** ART initiation was associated with increased CMV, but not HSV-2, shedding at weeks 4 and 8 after starting treatment. Mucosal shedding of CMV and HSV-2 was associated with different patterns of changes in systemic inflammatory biomarkers IL-6, CD14, CRP and TNFa.

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**Figure 1:** Heatmap of median cytokine value over time for CMV Ever-shedders (panel A) and Never-shedders (panel B) that had a baseline CD4 count of 200-1000 cells/µl.

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**323 HIV-1 Coinfection Skews iNKT Cells Toward Anergy and Exhaustion in People With Hepatitis C**

Danielle R. Nettene, Grant Williams, Joy Pickeral, Scott White, Cliburn Chan, Guido Ferrari, Susanna Naggie

Duke University School of Medicine, Durham, NC, USA, Duke University, Durham, NC, USA

**Background:** Hepatitis C virus (HCV) infection related liver disease remains a leading cause of morbidity and mortality in people with HIV (PWH). Liver fibrosis progression is more rapid in HIV/HCV coinfection compared to HCV monoinfection. Although direct acting antivirals (DAA) have improved treatment response substantially, access to DAA has declined since 2015, thus liver disease remains a threat to people with HIV/HCV. iNKT cells, a rare innate-like subset of T cells, may play a role in the rapid progression of fibrosis because of their liver tropism and due to the early loss of “pro-healing” CD4+ iNKT cells in early HIV infection. How this impacts long term iNKT subset diversity and functionality, and the role of iNKT in fibrogenesis in people with HIV/HCV is unknown.

**Methods:** To investigate the phenotypic and functional differences of iNKT cells in people with HIV/HCV coinfection, we consented persons with HIV (N=7), HCV (N=6), HIV/HCV (N=7), and healthy controls (N=6). All patients with HIV were virally suppressed on antiretroviral therapy and all patients with HCV had chronic active infection. We collected PBMCs and used 12-color flow cytometry to evaluate the phenotypes and functionality of iNKT following T-cell receptor (TCR) stimulation. Phenotypes were compared using Leiden clustering and dimension reduction.

**Results:** iNKT from PWH cells showed impaired expansion to TCR stimulation (~2 fold) compared to iNKT cells from persons with HIV or neither infection (up to 50-fold). The iNKT cells from PWH also showed a higher degree of TCR downregulation and lower CD38 expression following TCR stimulation, consistent with a more anergic state. We observed a higher frequency of CD8+ iNKT cells in PWH, which represents an effector subset. In addition, they were more likely to express CD57, a marker of terminal differentiation.

**Conclusion:** iNKT cells from PWH and people with HIV/HCV have impaired expansion, higher TCR downregulation in response to stimulation, and are biased towards “effector” and terminally differentiated subsets. These results suggest a picture of proinflammatory but relatively anergic iNKT cells.

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**324 Low SKAP1 Levels Indicate a Potential New Mechanism in HIV Immunological Non-Responders**

Wilhelm A. Voël, Jiang Kun, Jessica D. Santos, Albert L. Groenendijk, Marc Blaauw, Louise E. van Elk, Nadira Vadaq, Vasiliki Matzaraki, Mike van der Kooy, Willem L. Blok, Jannene E. Stalenhoef, Jan van Lunzen, Yang Li, Cheng-Jian Xu, Andre J. van der Ven

1 Radboud University Medical Center, Nijmegen, Netherlands, 2 Medizinische Hochschule Hannover, Hannover, Germany, 3 Erasmus University Medical Center, Rotterdam, Netherlands, 4 ViiV Healthcare, Brentford, United Kingdom, 5 UvA, Amsterdam, Netherlands

**Background:** Immunological non-responders (INRs) are people living with HIV (PLHIV) who fail to adequately restore CD4+ T-cell levels during suppressive ART. In contrast, immunological responders (IRs) do achieve CD4+ T-cell levels comparable to healthy individuals. Morbidity and mortality are increased in INRs. However, the pathogenesis of INRs is multifactorial and not fully elucidated. In this exploratory proteomics study, we looked for proteins which might be related to INR. SKAP1 is a lymphocyte protein which is important for T-cell migration, activation and proliferation. Previous work has shown that lymphocyte proliferation and IL-2 production were severely impaired in SKAP1 knock-out mice. The main function of SKAP1 is activating LFA-1, an integrin binding receptor. Knockdown of one of the LFA-1 heterodimers causes impaired CD4+ T-cell migration to the intestine and lymph nodes. SKAP1 has never been described in the context of HIV.

**Methods:** The 2000HIV study (clinicaltrials.gov NCT00394835) is a Dutch cross-omics multi-center study enrolling 1895 virally suppressed PLHIV, divided into a discovery and an independent validation cohort. We performed linear regression differential expression analysis on targeted serum proteomics (Olink, 2368 proteins) of INRs compared to IRs. In addition, we analyzed whole blood DNA methylation (NanoDrop spectrophotometer).

**Results:** The discovery cohort consists of 62 INRs and 1224 IRs, and the validation cohort of 26 INRs and 243 IRs. INRs were older and had more advanced HIV disease before starting cART. Proteomic analysis showed that SKAP1 was significantly downregulated in INRs in both the discovery (logFC = -0.545, FDR<0.0001) and the validation cohort (logFC = -0.628, pval=0.0001) (Figure 1A). SKAP1 was the most significant protein in both cohorts. In addition, we observed hypermethylation at cg26532208, a CpG site in the promoter area of the SKAP1 gene in the discovery cohort (logFC = -0.096, FDR = 0.042) (Figure 1B), as well as in the validation cohort (logFC = -0.093, pval=0.00078) which suggests decreased transcription. Finally, a significant correlation between CD4+ T-cell count and SKAP1 levels was found in the overall cohort irrespective of immunological responder status.

**Conclusion:** We found evidence for decreased SKAP1 expression levels in INRs in a large cohort study across two separate -omics layers. Previous work on SKAP1 function and these new data emphasizes its potential role in INR pathogenesis and might open new therapeutic avenues.
dissection of the discovery cohort. SKAP1 is downregulated in INRs.

Figure B: DNA methylation analysis of the discovery cohort shows hypermethylation in the promoter region of SKAP1 on chromosome 17 in INRs.

325 Disruption of Intestinal Germinal Centers During HIV Infection
Francesca Cossarini, Azra Khek, Pablo Canales-Herreries, Michael Tankelevich, Benjamin K. Chen, Judith A. Aberg, Francesca Petralia, Alexandros D. Polydorides, Saubhaj Mehandru
Icahn School of Medicine at Mt Sinai, New York, NY, USA

Background: HIV infection causes a profound dysregulation of the humoral immune system. Humoral responses occur mostly in secondary lymphoid tissue and involve the formation of Germinal Center (GC) and T-dependent antibody production results from interaction between cognate T and B cells. While the impact of HIV infection on mucosal T cells is well-reported, less is known regarding the effects of HIV on the humoral immune compartment. We investigated changes induced by HIV infection on humoral immunity, focusing on GC as the site of T-dependent antibody production in the gut-associated lymphoid tissue (GALT).

Methods: We prospectively enrolled people with HIV (18 with HIV viral load (VL)>1000 copies/mL and 46 with HIV VL<50 copies/mL on antiretroviral treatment (ART) as well as people without HIV (n=80) and examined intestinal immune cell changes with immunohistochemistry, flow cytometry and single cell RNA sequencing (scRNA seq).

Results: Immunohistochemical staining showed a significant decrease in GC size in participants with detectable VL compared to participants without HIV (median, IQR size 0.008 (0.0006–0.03) vs 0.1 (0.009–0.15) mm²; p=0.04), despite similar overall lymphoid follicle size. Additionally, we found a marked reduction of BCL6 expression in germinal centers in participants with detectable VL, even when accounting for the reduced GC size (556 (20–2375) vs. 4120 (3363-6400) cells/mm²; p=0.065). Using flow cytometry, in both ileum and colon, a significant depletion of GC B cells was observed in PWH with detectable VL compared to participants with undetectable VL and without HIV (0.07 (0.03–0.42) vs 0.4 (0.16–2.53) vs 0.88 (0.17–2.98) % of Live Cells in the ileum, p<0.008 and 0.39 (0.21–0.91) vs 0.75 (0.4–1.1) vs 1.04 (0.4–1.4) % of Live Cells in the colon, p=0.004). Within the scRNAseq dataset, cells mapping to GC B cells (BCL6, AICDA, CD38) were almost absent in participants with detectable VL at both sites. Differentially expressed gene analysis revealed increased expression of genes related to B cell activation and proliferation (MIF, CD74) in GC B cells from participants with HIV and suppressed VL and in genes related to migration of B cells to the GC (GNAI2, SWAP70) and T cell dependent B cell maturation (BAST1) in GC B cells from participants without HIV.

Conclusion: HIV infection is associated with a profound loss of intestinal GC B cells and may represent a major source of perturbation of humoral immunity at these sites of viral replication.

326 Persons With Residual Viremia on ART Have Higher Frequencies of Myeloid-Derived Suppressor Cells
Patrick Mehta, Evgenia Aga, Ronald J. Bosch, Hanna Mar, Deborah K. McMahon, Rajesh T. Gandhi, Joseph J. Eron, John W. Mellors, Charles R. Rinaldo, Bernard Macatangay, for the ACTG A5321 Team

University of Pittsburgh, Pittsburgh, PA, USA, Harvard TH Chan School of Public Health, Boston, MA, USA, Massachusetts General Hospital, Boston, MA, USA, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population that suppresses T, B, and NK cell function through multiple mechanisms and expand with acute and chronic inflammatory conditions. We evaluated whether frequencies of MDSCs are associated with measures of HIV persistence and levels of inflammation in persons with HIV (PWH) on long-term ART.

Methods: In the ACTG A5321 cohort of PWH with well-documented suppression of HIV-1 viremia on ART, we measured peripheral blood frequencies of classic (c) MDSC (Lineage(-), CD33+, HLADR-) and monocytic (m) MDSC (Lineage(-), CD33+, HLADR+, CD14+, CD11b+) by flow cytometry. To evaluate anti- or pro-inflammatory profile, we calculated the ratio of %mMDSC (anti-) and %intermediate monocytes (iMono; CD14+CD16+) pro-inflammatory). We then determined associations between MDSC frequencies and mMDSC/iMono (higher ratio – more anti-inflammatory) with measures of HIV persistence, including residual viremia, HIV DNA, cell-associated HIV RNA, and intact proviral DNA, and with soluble measures of inflammation.

Results: The 225 participants had a median age of 49 yrs, CD4 count of 681 cells/mm³, and ART duration of 7 yrs. The median cMDSC frequency was 0.13% of all live cells while mMDSC was 0.08%. Frequencies of both MDSC populations correlated with each other (r=0.31; p<0.001, Spearman). Lower mMDSC frequencies were associated with longer ART duration (r=-0.20; p<0.001). Participants with residual viremia (HIV RNA≥40 copies/mL) had higher %cMDSC than those with HIV RNA<40 copies/mL (0.14% vs 0.11%; p=0.016, Wilcoxon; Fig 1). There was also a trend for higher %mMDSC in those with residual viremia (0.09% vs 0.07%; p=0.05). Higher frequencies of mMDSC and cMDSC were associated with lower levels of pro-inflammatory cytokines IP-10 (r=-0.23, p=0.001) and TNF (r=-0.15, p=0.024) even after adjusting for pre-ART RNA. The mMDSC/iMono ratio did not correlate with any measure of HIV persistence. Higher ratios were associated with lower IP-10 (r=-0.24, p<0.001) and TNF (r=-0.15, p=0.028) levels even after further adjusting for age, pre-ART CD4 count, and years on ART.

Conclusion: Our finding that higher MDSC frequencies are associated with residual viremia despite long-term ART suggests that MDSC could inhibit virally suppressive HIV-specific immune responses. Reversing MDSC-mediated suppression would possibly enhance immune control of HIV but could also increase chronic inflammation.

327 Persistent HIV-1 Plasma Viremia in Patients on Therapy Caused by Macroophage-Tropic Lineage

University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, The Valley Health System, Las Vegas, NV, USA, Brigham and Women’s Hospital, Boston, MA, USA, Stony Brook University, New Orleans, LA, USA

Background: Antiretroviral therapy (ART) usually suppresses plasma HIV-1 RNA to levels of detection within months of initiation. Rarely, persistent low-level viremia occurs despite good ART adherence due to viral release from a...
large, clonally expanded reservoir of HIV-infected CD4 cells. We describe two PWH on ART with persistent, high-level viremia that failed to fully suppress for >10 months, and was associated with persistent macrophage-tropic virus.

**Methods:** Two PWH (NP1, NP2) initiated ART with high HIV-1 viral load and low CD4 counts. Both were adherent to ART regimen, by observation and drug levels and no evidence of resistance emergence by Sanger and deep sequencing. Plasma samples were collected from both participants, and total PBMCs from one. Viral RNA was sequenced using MiSeq with Primer ID on portions of pol and the env V1/V3 region. Near-full length sequencing was conducted on proviral DNA from PBMCs and viral RNA from the plasma. Full length env were cloned from plasma RNA and proviral DNA for pseudoviral assays to assess infection efficiency at low CD4 density as a predictor of macrophage tropism.

**Results:** No DRMs were detected in either participant and a diverse plasma RNA population was seen in the V1/V3 region. In NP1, a viral lineage representing ~50% of the total sequences rapidly declined, leaving a single, diverse persistent lineage, with no evidence of ongoing replication. In both participants, pseudoviruses generated from HIV envelopes of the persistent viremia were able to infect low CD4 density cells to a similar degree as other described macrophage tropic viruses. In NP1, the plasma viral lineage that disappeared with ART initiation and that was present in total PBMC DNA did not infect at low CD4 density, consistent with T cell tropic viruses. Sequencing of PBMC DNA in NP1 found 2% of proviruses recovered were of the macrophage tropic lineage. Viral integration site analysis suggested intact proviral DNA from PBMCs was not clonal. Curiously, in this participant all M tropic viruses were also found to have mutations in Vpr that abolished its open reading frame.

**Conclusion:** We describe two PWH with persistent high-level plasma viremia derived likely from infected macrophages with a long half-life, with additional studies being explored to confirm this. This has both clinical significance as the treatment regimens are still effective at preventing further infection, and has implications to the persistence of the reservoir in long-lived macrophages.

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**328 Variable HIV-1 C Env Characteristics Associated With Predicted bNAb Resistance in Botswana**

**Natasha O. Moraka**, 1 Wonderful T. Choga, 1 Marea Pemai, 1 Irene Gobe, 1 Margaret Makomane, 1 Onilametse T. Bareng, 1 Lynnette Bibleh 1, Molly Pretonus Holme, 1 Terence Hammard, 1 Catherine K. Koofhethile, 1 Joseph M. Makhemia, 1 Roger Shapiro, 1 Shahin Lockman 2, 1 Sukhilele Moyo, 3 Siamani Gasetiwe 1, 3 Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 3 University of Botswana, Gaborone, Botswana, 4 Harvard TH Chan School of Public Health, Boston, MA, USA

**Background:** Characterizing HIV-1 founder viruses is essential for informing vaccine design and broadly neutralizing antibody (bNAb) design. We analyzed variable region characteristics (VC) in HIV-1 serocorrelators and compared them with predicted bNAb resistance/sensitivity using machine learning techniques.

**Methods:** We analyzed HIV-1 near-full-length proviral sequences from 140 adults with documented recent HIV-1 serocorrelation who were enrolled in a previous population-based household study (BCPP, 2013-2018). We determined the variable loop (V1-V5) length and net charge patterns using the Variable Region Characteristics (VC) tool in the LANL HIV sequence database (hiv.lanl.gov). VC were stratified by bNAb resistance predicted using the bNAb-ReP algorithm (https://github.com/RedaRawi/bNAb-ReP). Wilcoxon ranksum test was used for assessment of variables between predicted bNAb resistance or sensitivity. We also assessed the presence of signature mutations associated with predicted resistance.

**Results:** A total of 140 consensus sequences were included in this analysis. Median log viral load (VL) of participants was 3.9 (Q1, Q3: 3.2, 4.4), median age 27 years (Q1, Q3: 22,33) and 20.7% were male. Few differences were observed between resistant and sensitive variants when looking at V1-V4 loop lengths, except for VRC26.25 and NIIH45-46 where V1 loop was shorter in resistant compared to sensitive strains (p<0.01 and p=0.04) respectively. The most significant differences in variable regions between resistant and sensitive strains were observed with V5 loop length; where CD4 bNAb ZG12, DH270.5 and FF interface 35022 resistant strains had shorter V5 loop lengths; p = <0.01, <0.01 and 0.02 consecutively. V1 charge distributions were significantly different for VRC26.25 (p = 0.01), PGT121 (p<0.01) and VRC01 (p = 0.01) resistant strains. In terms of mutations, E164 was observed and associated with resistance to both CH01 and PGT145. We observe the mutation T234N in sequences resistant to 3BNC1317 and b12. We did not record mutations N671T, W672L, W680G and F673L among all 25f resistant strains. We did however observe K683R mutation in all strains resistant to 25f.

**Conclusion:** Our findings further highlight the need to evaluate VC from sequence data, to determine the optimal vaccine design and best bNAb combinations to use for neutralization of highly variable HIV-1 subtype C.

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**329 Cannabis Use Enhances Mucosal Immunity and the Microbiome in Individuals With HIV**

**Robert Langat**, 1 Ryan K. Cheu, 2 Christopher Basting, 1 Courtney A. Broedlow, 1 Michael Luella, 1 Nina Isoherranen, 1 Ann C. Collier, 1 Peter W. Hunt, 1 Jennifer A. Manuzak, 1 Nichole R. Klatz 1

1 University of Minnesota, Minneapolis, MN, USA, 2 Emery Pharma, Alameda, CA, USA, 3 defeadHIV, Seattle, WA, USA, 4 University of Washington, Seattle, WA, USA, 5 University of California San Francisco, San Francisco, CA, USA, 6 Tulane University, Metairie, LA, USA

**Background:** Cannabis is widely used by people living with HIV (PLWH) both recreationally and to mitigate HIV- or antiretroviral therapy (ART)-associated nausea, pain, anorexia, or other symptoms. Here, we evaluated immune function and intestinal health among a cohort of ART-treated, cannabis-using, and non-using PLWH. We hypothesized that cannabis using PLWH would have reduced inflammation and immune activation linked with a more immunomodulatory gastrointestinal (GI) microbiome.

**Methods:** Colon single-cell suspensions from cannabis-using and non-using ART-treated PLWH were analyzed for cellular immune function by multiparameter flow cytometry. Plasma levels of short-chain fatty acids (SCFA) as indicators of colonic function were assessed by GC-MS. GI microbial communities were profiled through colonic mucosa 16s rRNA sequencing. Cannabis use or non-use was verified in all participants by LC-MS.

**Results:** Although overall frequencies of CD4+ and CD8+ T-cells were unchanged (p=0.692 and p=0.204, respectively), the frequencies of activated HLA-DR+CD38+ CD4+ and CD8+ T-cells were significantly decreased in the colon of ART-treated cannabis-using as compared to non-using PLWH (p<0.0001 for both subsets). Frequencies of colon IgA+ and IgG+ B cells were significantly increased in cannabis users vs. non-users (p=0.0006 and p=0.0259, respectively). Cannabis users had significantly lower frequencies of colon TNF-α+CD20+B cells as compared to non-users (p=0.0001). Plasma concentrations of the SCFAs acetate (p<0.0001), propionate (p=0.0034), butyrate (p=0.0001), and isobutyratic acid (p=0.0043) but not valeric acid and isovaleric acid were increased in cannabis users vs. non-users. Lastly, ART-treated cannabis using PLWH possessed colonic mucosa microbiomes dominated by Prevotella, followed by Bifidobacterium, Faecalibacterium, Succinivellulimonas, and Collinsella. No differences in alpha diversity were observed between ART-treated cannabis-using and non-using PLWH.

**Conclusion:** ART-treated cannabis using PLWH had significantly lower frequencies of activated CD4+ and CD8+ T-cells and TNF-α+CD20+B cells, suggesting lower inflammation and immune activation as compared to non-cannabis users. Cannabis use has the potential to alleviate HIV-associated nausea, pain, anorexia, or other symptoms. Here, we evaluated immune function and intestinal health among a cohort of ART-treated, cannabis-using, and non-using PLWH. We hypothesized that cannabis using PLWH would have reduced inflammation and immune activation linked with a more immunomodulatory gastrointestinal (GI) microbiome.
330 HIV, Not Integrase Inhibitor-Based ART, Modifies Gut Microbiota Composition in MSM
Marta Rosas Cancio-Suárez, Luis Miguel Nieto-Salas, Claudio Díaz-García, Jorge Díaz-Alvarez, Alejandro G. García-Ruiz De Morales, Clara Crespillo Andújar, Laura Luna García, Elena Moreno, Laura Martín-Pedraza, María Fons, Javier Martínez-Sanz, Raquel Ron, Santiago Moreno, Matilde Sánchez-Conde, Sergio Serrano-Villar
Hospital Ramón y Cajal, Madrid, Spain
Background: Understanding gut microbiota variations in HIV is confounded by factors like antiretroviral therapy (ART), immune status, and sexual orientation. We aimed to isolate the impact of integrase inhibitor (INSTI)-based ART on gut microbiota among men who have sex with men (MSM) across varying conditions of HIV status and immune recovery.
Methods: We conducted an observational study with three cohorts: 1) MSM HIV-negative (HIV-) initiating post-exposure prophylaxis (PEP) with raltegravir, tenofovir disoproxil fumarate, and emtricitabine, sampled on days 0 and 28. 2) MSM with advanced HIV (HIV+ <350 CD4+ T-cells), sampled before and after 48 weeks after starting INSTI-based ART. 3) MSM with HIV and adequate immune recovery (HIV+ >500 CD4) after an average of 9.5 years on ART. Stool samples were processed for DNA extraction, sequenced for the V3–V4 regions of the 16S rDNA gene using the Illumina platform and analyzed through the QIIME2 workflow. DADA2 was used to infer Amplicon Sequence Variants, which were assigned to taxonomic entities up to the genus level with a Silva-based 16S Naive Bayes classifier. Differential abundance testing to identify specific taxa was executed with ANCOM.
Results: We included 22 MSM HIV-, 23 MSM HIV+ <350 CD4, and 29 MSM HIV+ >500 CD4 participants. Pairwise comparisons were performed to delineate the effects of ART, HIV status, and immune function on microbiota composition. Alpha diversity varied significantly between specific groups (Figure 1A), with highest values in HIV- MSM compared to HIV+ MSM. Weighted UniFrac analysis also demonstrated microbial composition differences between MSM with and without HIV (Figure 1B). Lachnoclostridium genera was more abundant in MSM HIV+ < 350 CD4 (p = 0.0017). Alloprevotella was significantly higher in MSM without HIV (Figure 1B). Lachnoclostridium genera was more abundant in MSM with and without HIV (Figure 1B).
Conclusion: In this study not confounded by sexual orientation, HIV and immune status significantly affected gut microbiota composition. However, we found no evidence to suggest that INSTI-based ART negatively influences gut microbiota composition.

Figure

A

B

331 Microbe-Drug Interactions Between Antiretrovirals and the Gut Microbiome in HIV Infection
Christopher Basting, Ty Schroeder, Adrian Velez, Courtney A. Broedlow, Timothy Griffin, Candace Guerrero, Nichole R. Klatt
University of Minnesota, Minneapolis, MN, USA
Background: For people living with HIV (PLWH), antiretroviral therapy (ART) typically controls HIV replication and greatly reduces mortality, however, does not cure HIV and PLWH must maintain ART treatment inevitably. During passage through the GI tract, ARVs encounter the gut microbiome, where they may affect the growth of microbes or potentially be metabolically transformed by bacteria into less active or toxic forms. Here, we investigated the antimicrobial effects of 17 different ARVs and their in vitro ability to alter microbes to better understand how ART may impact the gut microbiome in PLWH. Furthermore, we investigated the ability of gut bacteria to metabolize ARVs to understand if the gut microbiome may play a role in bioavailability of ARVs.
Methods: Bacterial isolates and stool communities were grown from glycerol stock and diluted into fresh media containing the selected ARVs. Growth of bacterial isolates was monitored by measuring the OD600 every 30 minutes for 48 hours. Changes in the bacterial stool communities was determined by 16S V4 rRNA sequencing after 24 hours of incubation with ARVs. ARV drug metabolism by bacterial isolates and whole stool communities was determined by measuring drug concentrations by LC/MS/MS utilizing a novel assay developed in our lab to concurrently measure 17 ARVs.
Results: ARVs, especially the integrase inhibitors dolutegravir and elvitegravir, significantly inhibit the growth of gut bacteria including Bacteroides fragilis and Prevotella stercorea (p < 0.05, Welch’s t-test). The total bacterial load of in vitro stool cultures measured by the 16S region was significantly different between drug treatments (p < 0.05, Kruskal-Wallis). Furthermore, bacterial stool communities from HIV-infected individuals significantly depleted tenofovir disoproxil fumarate (p = 0.005, Welch’s t-test) and cultures with bacterial isolates including Bacteroides fragilis depleted elvitegravir and cobicistat (p = 0.0102 and p = 0.0108 respectively, Welch’s t-test).
Conclusion: Overall, these results show that specific ARVs can inhibit the growth of strains of gut bacteria and have an impact on a bacterial community’s composition in vitro. This has potential implications in how ART affects the gut microbiome of PLWH and associated microbial metabolic products, which is under further study in the lab. Finally, bacterial communities from stool have the metabolic potential to deplete specific ARVs in vitro, which may be important in the interindividual success of ART for PLWH.

Aya Ishizaka, Michiko Koga, Taketoshi Mizutani, Hiroshi Yotsuyanagi
University of Tokyo, Tokyo, Japan
Background: The gut microbiota of PLWH who have achieved good immune recovery through ART is as diverse as that of healthy individuals, but the composition of the bacterial taxa is significantly different, which is indicated to be related to chronic inflammation. We evaluated the correlation between changes in the gut microbiota, clinical information, and inflammation biomarkers in PLWH between baseline and after 4 years of follow-up.
Methods: Stool and blood samples, along with the laboratory parameters, were collected from 46 PLWH at baseline and after 4 years of follow-up. The microbiome was characterized by sequencing of the 16S rRNA V3–V4 regions on the Illumina MiSeq platform, and data were analyzed using QIIME2 software. Functional gene predictions of the bacterial microbiota were inferred from the 16S rRNA using PICRUSt2 pipeline. The concentrations of cytokine and LPS-binding protein (LBP) were quantified using a Bio-Plex System and ELISA, respectively.
Results: There were no significant changes in laboratory parameters between baseline and follow-up except for body mass index (BMI). However, the progression of gut dysbiosis was observed; Lachnospiraceae, involved in short-chain fatty acid (SCFA) production, decreased, while Enterobacteriaceae, potentially pathogenic bacteria, increased. A decrease in vitamin B1 (thiamine) biosynthesis was predicted from 4-year alternations, suggesting an intestinal environment that is less conducive for SCFA-producing bacteria to proliferate. Higher levels in IL-27, IFN-β (an LPS/TLR4-inducible cytokine) and IL-8 at follow-up suggested the progression of intestinal permeability while HIV RNA remained low on ART. These cytokines were positively correlated with the bacterial translocation measured by plasma LBP. An increased BMI was significantly associated with IL-16 and CXCL13, both of which are involved in intestinal inflammation and bacterial migration. A low abundance of Parabacteroides which lead to the disturbances of secondary bile acid metabolism, was associated with a BMI of 25 and over, low a diversity, low abundance of SCFA-producing bacteria, as well as weight gain for 4 years.
Conclusion: HIV-specific dysbiosis progressed despite effective ART. This dysbiosis correlated with weight gain and was characterized by Intestinal
permeability that also persisted and was associated with systemic inflammation. This suggests that the intestinal environment in PLWH may potentially affect metabolic imbalance and inflammation.

**Panel A** Comparative analysis of inflammatory cytokine levels in PLWH between baseline and follow-up. **Panel B** Correlation analysis between gut microbiota (genus) and BMI. *p* < 0.05, **p** < 0.01, ***p*** < 0.001

### Comparisons of Oral and Gut Bacteria Highlight Role of *Veillonella* in Systemic Inflammation in HIV

Grace Cho, Julie Elliott, Katharine Newman, Fan Li, Nicole H. Tobin, Steven Shoptaw, Pamina Gorbach, Grace M. Aldrovandi, Jennifer A. Fulcher

**Background:** Chronic inflammation contributes to multiple comorbidities in people living with HIV. Translocation of gut bacteria and resultant immune activation may in part drive this inflammation; however, the role of the oral microbiome in this phenomenon is unknown. We investigated the effects of HIV on the oral microbiome and the relative contribution of oral and gut bacteria to systemic inflammation.

**Methods:** Oral and gut microbiome composition was determined by 16S rRNA gene sequencing of saliva samples and rectal swabs from 98 men who have sex with men ( MSM) with HIV and 99 MSM without HIV. Biomarkers were quantified in serum using multiplex Lumine assays. Relative contributions of oral versus gut bacteria to biomarker variation was analyzed using PERMANOVA and significant relationships identified using mixOmics. Anti-bacteria IgG ELISAs were developed to assess oral bacteria translocation and quantified by serum endpoint IgG titer. Relationships between immune biomarkers and endpoint IgG titers were examined using correlation analysis.

**Results:** The oral microbiome composition in MSM differed by HIV status with increased *Veillonella*, *Capnocytophaga*, and *Megasphaera* seen with HIV. Known inflammatory biomarkers also increased with HIV (fatty acid binding protein 2 (FABP2) p<0.001; sCD27 p=0.006; sCD163 p=0.01; CXCL10 p<0.001; IL-6 p=0.03; TNF-alpha p=0.007). The oral microbiome was an important contributor to inflammatory biomarker variance showing greater influence than the gut for several biomarkers (sCD27, LPS-binding protein (LBP), CRP, CXCL10, TNF-alpha, IL-6) (Figure). Anti-bacteria (*Veillonella parvula*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*) endpoint IgG titers did not differ by HIV status. However, there was a significant correlation between both LBP (r = 0.34, p<0.02) and sCD14 (r = 0.3, p=0.04) with *Veillonella parvula* IgG in MSM with HIV. mixOmics analysis also identified key correlations between oral *Veillonella* with IL-6 and FABP2. In vitro studies showed heightened inflammatory potential of *Veillonella parvula* with peripheral immune cells.

**Conclusion:** The oral microbiome differs in MSM with HIV and is an important contributor to altered inflammatory biomarkers. Correlations between key oral bacteria and inflammatory biomarkers suggest that systemic circulation of oral bacteria, including *Veillonella parvula*, could be another important mechanism driving chronic inflammation in HIV.

Figure: Relative contribution of oral vs gut bacteria to inflammatory biomarker variance as determined using PERMANOVA. Percentages represent the percent variance explained by each microbiome compartment.

### CX3CR1+ V61 T-Cells Are Clonally Expanded and Driven by CMV, Microbiota, and HIV-1 in the Gut on ART

Nived Collercandy, Camille Vellas, Manon Nayrac, Mary Requena, Thomas Richarme, Justine Latour, Karl Barange, Laurent Alric, Nicolas Carrere, Guillaume Martin-Blondel, Matteo Serino, Jacques Iotop, Pierre Delobel

**Background:** The V61 subset of γδ T cells resides mainly in tissues, where it participates in innate and adaptive immunosurveillance, notably in the gut, a major site for HIV-1 persistence. V61 cells normally represent a minor part of blood γδ T cells, but they expand during HIV-1 infection, with an inversion of the V61/V52 ratio, the causes of which are not yet clear.

**Methods:** Duodenal biopsies and blood samples from 15 virologically suppressed people living with HIV-1 (PLWH) and 15 uninfected controls recruited in the ANRS EP61 GALT study were used to assess the frequency, phenotype and function of V61 T cells by FACS. Samples from 5 PLWH and 5 controls were used to sequence the TRDV1 chain repertoire. Single-cell RNA-seq was performed on sorted circulating V61 T cells from 6 PLWH and 6 controls. Total and intact HIV-1 DNA and residual HIV-1 RNA were quantified, and 16S RNA PCR was used to amplify and sequence bacterial DNA in gut and blood samples. All selected subjects were CMV seropositive.

**Results:** PLWH had increased circulating CX3CR1+ V61 effector cells compared with uninfected controls (median 17.2/μL, [4.5-26.3] vs. 3.3/μL [1.2-7.3] respectively; P=0.03). The phenotype of expanded CX3CR1+ V61 cells globally clustered with terminal differentiation markers (TEMRA: CD27-CD45RA+), and into subpopulations expressing the activating receptor NKG2C and the exhaustion marker TIM-3. CX3CR1+ V61 TEMRA cells were highly cytotoxic, with high levels of granzyme B and perforin, but low production of IFNγ and TNFα. Gut intra-epithelial lymphocytes (IEL) were more cytotoxic (perforin+) and activated (NKG2C+) in PLWH, the latter correlating with the V61/V52 ratio in blood. Repertoire analysis revealed clonal expansions in PLWH blood, compared with matched duodenal IELs and uninfected controls. The expansion, phenotype, and cytotoxicity of blood V61 cells in PLWH were notably associated with the CMV IgG index, the abundance of various bacterial species in the duodenal and blood microbiome, and HIV-1 RNA and DNA in gut and blood samples (Figure).

**Conclusion:** Highly cytotoxic CX3CR1+ V61 effector cells are clonally expanded in the blood and associated with V61 IEL activation in the gut of PLWH on ART. They may be triggered by the interplay between residual CMV replication, gut and blood microbiome, and the gut HIV-1 reservoir. CX3CR1+ V61 effector cells may contribute to the control of HIV-1 persistence on ART, but might also be involved in chronic vascular inflammation in PLWH due to their endothelial tropism.
Bacteria-Induced Granzyme B Contributes to HIV-1-Mediated Gut CD4 T-Cell Depletion Ex Vivo

Kaylee Mickens, Stephanie M. Dillon, Kejun Guo, Ashley Thompson, Bradley S. Barrett, Mario L. Santiago, Cara C. Wilson
University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Background: The gastrointestinal (GI) tract is a major site for early, massive, and persistent CD4 T cell depletion following acute HIV-1 infection. The mechanisms driving this profound death of gut CD4 T cells remain poorly understood. Among persons with HIV-1 (PWH), a disrupted epithelial barrier results in the translocation of bacteria from the gut lumen to the underlying lamina propria (LP) and systemic circulation. We reported that ex vivo exposure of LP mononuclear cells (LPMC) to enteric bacteria augmented HIV-1-mediated gut CD4 T cell death, shifting the death mechanism from pyroptosis to apoptosis. Microbial exposure of gut CD4 T cells ex vivo upregulated granzyme B (GZB)—an enzyme that facilitates target cell killing by cytotoxic T/NK cells via apoptosis. GZB+ CD4 T cells were detected at higher frequencies in colon biopsies of PWH compared to uninfected controls, despite lower overall CD4 T cell frequencies.

Methods: To test if GZB plays a critical role in HIV-1-mediated gut CD4 T cell death, LPMC (n=6 donors) were infected with Transmitter/Founder (TF) HIV-1 (CH040) or mock then exposed to bacterial lysate (Escherichia coli) in the presence/absence of a specific GZB inhibitor, Z-AAD-CMK. 4dp LPMC were collected and GZB expression, infection (HIV-1 p24), apoptosis (AnnexinV, viability dye/AqVi), and number of CD4 T cells were determined by flow cytometry. 0X40 and TNFR2 expression were assessed by flow cytometry.

Results: Higher levels of TF HIV-1 infection were observed in GZB+ versus GZB- CD4 T cells (3-fold, p=0.003). GZB+ CD4 T cells showed 1.56-fold higher levels of apoptosis markers (AqVi− AnnexinV+) compared to GZB- cells (p=0.007). TF HIV-1 infection led to substantial CD4 T cell death compared to mock—on average 44% of CD4 T cells depleted in TF HIV-1 infected cultures (p=0.01). Z-AAD-CMK inhibited GZB activity in E. coli-stimulated CD4 T cells by 64%; GZB inhibition rescued CD4 T cells from HIV-1-mediated death (16% vs 44% depletion; p=0.02). HIV-1-infected GZB+ CD4 T cells that survive to 4dp showed higher expression of anti-apoptotic factors 0X40 and TNFR2 compared to GZB- cells (p<0.05).

Conclusion: Our findings suggest that bacteria-mediated GZB induction may be a mechanism contributing to massive CD4 T cell death in the GI tract during acute HIV-1 infection. The induction of anti-apoptotic factors in GZB+ CD4 T cells that survive in vitro HIV-1 infection raise the possibility that surviving GZB+ CD4 T cells in the gut may serve as a significant HIV-1 reservoir.

Distinct Intestinal Microbial Signatures Linked to Accelerated Biological Aging in People With HIV

Shalini Singh1, Leila B. Giron, Malha W. Shaikh, Shivanjali Shankaran, Phillip A. Engen1, Zlata R. Bogin, Simona A. Bambi2, Aaron Goldman, Toshitha Kannan, Ceylan E. Tanes3, Kyle Bittinger2, Alan Landay, Michael J. Corley, Ali Keshavarzian, Mohamed Abdel-Mohsen2

1Wistar Institute, Philadelphia, PA, USA, 2Rush University, Chicago, IL, USA, 3Children’s Hospital of Philadelphia, Philadelphia, PA, USA, 4Weill Cornell Medicine, New York, NY, USA

Background: HIV infection disrupts the intestinal barrier, resulting in persistent inflammation, even with antiretroviral therapy (ART). This inflammation contributes to aging-related comorbidities in people with HIV (PWH). However, it remains unclear whether ART-suppressed HIV affects intestinal biological aging and whether microbial dysbiosis and translocation contribute to aging in PWH on ART.

Methods: Colon and ileal biopsies, blood, and stool were collected from 25 PWH on ART (viral load <50 copies/ml) and 23 age, sex, and ethnicity-matched HIV-negative controls. Accelerated biological aging in colon, ileum, and blood was assessed by regressing biological age estimated by several epigenetic aging clocks (Horvath1, Horvath2, Hannum, PhenoAge, GrimaAge, and DunedinPACE) against chronological age. Intestinal integrity was assessed by immunofluorescence staining for tight junction proteins (ZO1, occludin). Markers of microbial translocation (e.g., LBP) and infection were measured by ELISA/multiplex arrays. Microbiota profiles of stool, ileum, and colon were determined via 16S rRNA sequencing, and metabolic analyses of plasma and stool were conducted using mass spectrometry.

Results: Despite similar chronological age (Fig. 1A), PWH exhibited accelerated biological aging of the ileum, colon, and blood. Colon and ileum from PWH showed reduced tight junction proteins and increased microbial translocation, significantly associated with accelerated biological aging and higher inflammation (P<0.05). Putative pro-inflammatory bacteria like Catenibacterium and Prevotella 2/9 were enriched in PWH, correlating with accelerated aging (FDR<10%). Conversely, short-chain-fatty-acid-producing and anti-inflammatory bacterial genera, like Subdoligranulum and Erysipelotrichaceae UCG-003 were depleted in PWH, correlating with decelerated aging. Correlation networks revealed associations between specific microbial genera in the colon and ileum (not shown) with accelerated aging, enrichment of pro- inflammatory microbial-related metabolites, and depletion of anti-inflammatory metabolites (P<0.05).

Conclusion: Distinct microbial profiles are linked to intestinal and systemic biological aging in PWH on ART. Further research is needed to understand the mechanisms connecting microbial dysbiosis/translocation to intestinal and systemic biological aging in PWH and to develop preventive strategies. The figure, table, or graphic for this abstract has been removed.

Deep Metabolic Profiling for Tissue-Specific Responses Against SARS-CoV-2 Variants in Hamster Models

Urvinder Kaur Sardarni1, Anoop Ambikan1, Arpan Acharya2, Samuel D. Johnson, Rajesh Rajaiah1, Kabita Pandey1, Ujjwal Neogi, Siddapapa N. Byrareddy3
1University of Nebraska Medical Center, Omaha, NE, USA, 2Karolinska Institute, Stockholm, Sweden

Background: While most people recover from acute COVID-19, a significant percentage experience long-term health issues known as post-acute sequelae of SARS-CoV-2 infection (PASC). The underlying host tissue response during acute infection might contribute to long-term effects in people with PASC. To understand this, we investigated the host-tissue responses of SARS-CoV-2 variants using the hamster model.

Methods: Syrian golden hamsters (SGHs) were infected with the delta and omicron variant, and uninfected SGHs were used as controls. The hamsters were euthanized four days post-infection, plasma and tissue samples from the lung, brain, heart and kidney were collected to analyse SARS-CoV-2 viral load and targeted metabolomics.

Results: Compared to omicron-infected SGHs, delta-infected SGHs had a higher viral load in lungs (p=0.02, heart (p=0.009), brain (p=0.019), and plasma (p=0.0007). However, viral load in the kidney of delta- and omicron-infected SGHs did not differ significantly. Principal component analysis (PCA) identified distinct brain metabolite profiles, while showing no disparity in heart metabolites between three groups. Kidney metabolites in infected SGHs differed from naive ones, and plasma metabolites in omicron-infected and naive SGHs were distinct from delta-infected SGHs. Detailed investigation of individual metabolites showed the varied effect of delta and omicron variants across multiple tissues. Delta variant infection led to differential regulation of 46, 34, 60, 165, and 37 metabolites, whereas omicron variant infection resulted in 21, 112, 67, 160 and 4 differentially regulated metabolites in lung, brain, heart, kidney and plasma respectively. In the brain and heart, major distinctions were observed in amino acids, with arginine, aspartate, methionine, proline, and tyrosine levels being higher in omicron-infected SGHs compared to those infected with the delta variant suggesting the dysregulation of amino acid biosynthesis/metabolism, nucleotide metabolism and energy metabolism pathways.

Conclusion: Our findings indicate that SARS-CoV-2 mediated tissue insult leads to altered host metabolites during acute infection in a strain specific manner. Therefore, our data provide a basis for understanding tissue responses during
Markers of Microbial Translocation and Inflammatory Cytokines Are Predictive of Severe COVID-19

Ty Schroeder, Christopher Basting, Kathie G. Ferbas, Adrian Velez, Courtney A. Broedlov, Erik Swanson, Melissa Bailey, Robert Langat, Luca Schifanella, Grace M. Aldrovandi, Nicole H. Tobin, Otto Yang, Jennifer Fulcher, Nicole R. Kliatt

University of Minnesota, Minneapolis, MN, USA, University of California Los Angeles, Los Angeles, CA, USA, National Institutes of Health, Bethesda, MD, USA

Background: The novel SARS-CoV-2 virus caused the global COVID-19 pandemic resulting in approximately 770 million global cases and 7 million deaths to date. Several factors predict severe COVID-19 including comorbidities such as age, cardiovascular disease and diabetes. Furthermore, gastrointestinal symptoms and microbial dysbiosis are commonly observed in COVID-19 and may therefore be indicators of severe disease. In this study, we aimed to understand the differences in markers of microbial translocation and circulating cytokines between healthy individuals and patients with severe COVID-19. We then investigated the accuracy of these biological factors in predicting whether an individual was healthy or had severe COVID-19.

Methods: A cohort of California-based participants were entered into the study, consisting of 62 patients hospitalized with COVID-19 and 115 healthy individuals. Plasma samples were used to measure circulating concentrations of cytokines by Luminex and markers of microbial translocation including LPS-binding protein (LBP), soluble CD14 (sCD14), intestinal fatty acid binding protein (I-FABP), and Zonulin were measured by ELISA.

Results: Hospitalized COVID-19 patients had significantly higher plasma concentrations of markers of microbial translocation including LBP and sCD14 compared to healthy individuals (p=1.4-17, p<9.35-5). Hospitalized patients also had elevated inflammatory plasma cytokine concentrations, most notably IL-6, TNF-α, IFN-γ, and IL-18. Spearman correlation analysis showed that IL-6 was significantly positively correlated with markers of microbial translocation including LBP and sCD14 (r=1.42-25, p=1.455-4). A random forest model showed the best accuracy in predicting healthy versus hospitalized individuals with a true positive percent (TPP) of 94%. LBP and IL-6 were the greatest drivers of prediction. This model was further tested on two other COVID-19 datasets with varying accuracy: 81% TPP and 75% TPP.

Conclusion: Hospitalized individuals were characterized by elevated inflammatory cytokines (especially IL-6) and microbial translocation measured by sCD14 and LBP. The correlation between inflammatory cytokines and markers of microbial translocation suggests a relationship between gut barrier integrity and systemic inflammation in COVID-19, which may better predict whether an individual is healthy or has severe COVID-19, with IL-6 and LBP being the most important variables in the prediction.

Complement-Driven Type-I IFN Response Enhances mTOR Activation and T-Cell Immunity

Marta Bermejo Jambrina, John L. van Hamme, Lieve van der Donk, Doris Wilflingseder, Teunis B. Geijtenbeek

VUMC, University of Minnesota, Minneapolis, MN, USA; University of Michigan, Ann Arbor, MI, USA; Academic Medical Center, Amsterdam, Netherlands

Background: Uncontrolled SARS-CoV-2 infection is associated with disorders of the innate immune and delayed adaptive immune systems. Yet, it remains unclear how SARS-CoV-2 causes local and systemic dysregulation. The role of the complement system in SARS-CoV-2 pathogenesis is well established, in particular as a driver of systemic inflammation which is characteristic of severe COVID-19. Although complement is traditionally known as a central arm of innate immunity, it has equally important roles in the regulation of adaptive immunity. However, how mechanistic complement affects dendritic cell (DC) functionality and T cell- priming upon SARS-CoV-2 infection, is still poorly defined.

Methods: Effects of differentially opsonized SARS-CoV-2-loaded primary DCs were measured by RT-PCR, ELISA, PathScan and flow cytometry. Co-culture with naive CD4+ and CD8+ T cells, T-cell activation, cytotoxic T-cells (GRZB/PRF1) production and SARS-CoV-2-specific cytokine-producing T-cells were measured by flow cytometry and ELISpot.

Results: DC were not susceptible to SARS-CoV-2 infection, and exposure to SARS-CoV-2 triggered neither induction nor secretion of type-I IFN and inflammatory cytokines. Notably, complement-opsonized SARS-CoV-2 loaded-DCs displayed enhanced maturation and efficient type-I IFN and IL-18 responses, suggesting that complement is crucial for mediating immunity against SARS-CoV-2. Here, we describe that IL-1β secretion occurs following intracellular caspase-1 activation by inflammasome activation, revealing a mechanistic link between complement and IL-1β secretion in human DCs. Strikingly, complement-triggered IL-1β production was mediated by the mammalian target of rapamycin (mTOR). NLRP3 inhibition resulted in impaired priming of IFN-γ-gamma-producing CD4+ and CD8+ T cells, suggesting an essential role of complement in increasing antigen-specific T-cell responses.

Conclusion: The potential for myeloid cells to act as bona fide targets of SARS-CoV-2 infection remains unclear. Here, we show that complement-opsonized SARS-CoV-2 induces type I IFN secretion, upregulates the mTOR pathway and directly activates NLRP3, which leads to IL-1β secretion. The role of IL-1β as part of the bridge between innate and adaptive immunity may be clinically translated into therapeutic strategies to empower the formation of T cell immunity. Our data demonstrate distinct immunological functions for DCs and consider the role of complement and mTOR activation in regulating immune system responses in SARS-CoV-2 infection.
Prolonged SARS-CoV-2 Viral Burden and Impaired Immunity in SIV/ SARS-CoV-2 Coinfected Macaques

Megan N. Fredericks1, Hannah Frizzell1, Hillary Tunngal1, Cecily C. Midkiff1, Jeana Barrow1, Anthony L. Cook2, Robert V. Blair3, Ankur Sharma4, Deborah R. Fuller5, Megan O’Connor6

1University of Washington, Seattle, WA, USA, 2Juliana National Primate Research Center, Covington, LA, USA, 3Washington National Primate Research Center, Seattle, WA, USA, 4Brinque, Inc, Rockville, MD, USA

Background: People living with HIV (PLWH) have increased risk of morbidity and mortality from COVID-19. SARS-CoV-2 infection in PLWH poses a risk of prolonged infection and viral shedding, and emergence of variants of concern. Using the SIV macaque model for AIDS, we test the hypothesis that immune dysfunction during HIV infection alters SARS-CoV-2 viral infection and COVID-19 disease.

Methods: Eight female rhesus macaques were intravaginally infected with SIVmac251, then intranasally and intratracheally inoculated with SARS-CoV-2 (WA-1) at 17-34 weeks post-SIV inoculation. Blood, bronchoalveolar lavage, stool, nasal, oral, and rectal swabs were collected pre-infection through 14 days post-infection (DPI). ELISA, ELISPOT, qRT-PCR, lung pathology, cytokine multiplex, and virus neutralization assays were performed to measure viral loads, pathogenesis, and immune responses.

Results: We observed no significant changes in SIV clinical symptoms or viremia post-SARS-CoV-2 co-infection, and COVID-19 disease was typically mild. At 14 DPI, SARS-CoV-2 replication persisted in the upper, but not the lower respiratory tract. Notably, SARS-CoV-2 RNA levels in nasal swabs were significantly higher in SIV+/SARS-CoV-2 infected rhesus when compared to published data in SIV-/SARS-CoV-2 rhesus (PMCID: PMC8462335, PMC829873). Anti-SARS-CoV-2 binding antibodies in sera were significantly lower in SIV+ macaques when compared to those from historical SIV-/SARS-CoV-2 infected controls. Furthermore, generation of SARS-CoV-2 spike-specific T-cell responses were hindered at 14 DPI.

Conclusion: Here, we provide evidence for the utility of the rhesus macaque in modeling human HIV/SARS-CoV-2 co-infection. Our results suggest that HIV-induced immunosuppression impairs generation of anti-SARS-CoV-2 immunity, that may in turn, contribute to prolonged SARS-CoV-2 viral shedding, increased transmission windows, altered disease pathogenesis, and lower protection against subsequent SARS-CoV-2 exposures. Studies in progress will determine whether SARS-CoV-2 viral evolution is accelerated in SIV-infected macaques.

Impact of COVID-19 Progression in Patients With Hypertension

Alba Sanchez1, Silvia Chafino1, Graciano Garcia-Pardo1, Frederic Gomez-Bertomeu2, Miguel Lopez-Dupla3, Antoni Del Pino-Rius4, Montserrat Ollona4, Francesc Vidal5, Anna Rull6, Joaquim Pereira6

1Hospital Universitario de Tarragona Joan XXIII, Tarragona, Spain, 2Rovira i Virgili University, Tarragona, Spain

Background: Coronavirus disease (COVID-19) due to SARS-CoV-2 infection is aggravated by some comorbidities, with arterial hypertension being one of the most common. Hypertensive patients are more vulnerable to developing severe COVID-19 complications through different joint mechanisms. The main objective is to find biomolecules and metabolic pathways through a multiomic approach that may explain the relationship between hypertension and unfavourable COVID-19 disease progression.

Methods: One hundred and three patients with COVID-19 were classified according to disease severity (mild, severe and critical) and divided into hypertensive (n=26) and non-hypertensive (n=77). Serum samples were collected at the time of admission (acute phase) and four to eight weeks later (recovery phase), and multiomics studies of proteins, lipids and metabolites were performed. Statistical analyses were carried out by Metaboanalyst 5.0 and SPSS Statistics 25.0.

Results: Hypertension was present in 25.2% of COVID-19 patients as a previous comorbid disease. Hypertension was significantly related to COVID-19 severity (p=0.008), in fact, 84.6% of the hypertensive patients belonged to the unfavourable COVID-19 group (severe and critical). The profile of biomolecules changed depending on the study phase, with the number of significant molecules in the acute phase (n=43) greater than those in the recovery phase (n=25). In the acute phase, myo-inositol (AUC=0.695), phosphatidylcholine 32.1 (AUC=0.643) and gelsolin (AUC=0.698) were best able to distinguish between hypertensive and non-hypertensive patients and in the recovery phase, gelsolin (AUC=0.674), zinc-alpha-2-glycoprotein (AUC=0.711) and octanoic acid (AUC=0.699) presented the best discriminatory power. Notably, relative abundance (mean±SD) of gelsolin decreased with COVID-19 severity (mild n=1.02±0.622, unfavourable n=0.763±0.367) (p=0.002) but increased in unfavourable patients with hypertension (0.919±0.388) compared to non-hypertension (0.681±0.332) (p=0.03) in the acute phase.

Conclusion: The results confirmed hypertension as a frequent comorbid disease in patients with unfavourable COVID-19 progression, which significantly increases the severity of the disease. Gelsolin, myo-inositol and lipids were those molecules that could be determinants in COVID-19 progression and could explain the clinical worsening of COVID-19 patients through different pathways such as immune system processes, inflammation, oxidative stress and lipid metabolism.

SARS-CoV-2 Evolution, Reinfection, and Sustained Viremia in Cancer Patients

Juliana D. Siqueira1, Livia R. Goes1, Brunna M. Alves1, Marianne M. Garrido1, João P. Viola1, Marcelo A. Soares2

1Instituto Nacional de Cancer, Rio de Janeiro, Brazil

Background: Several studies with longitudinal follow-up on SARS-CoV-2 infection have been reported. Most of them focus only on cases with suspected reinfection or prolonged infection in immunosuppressed individuals. Herein, we describe 26 different cases of SARS-CoV-2 infections in cancer patients with multiple longitudinal samples analyzed and described the different scenarios of sustained viremia, reinfection and viral evolution.

Methods: Cancer patients followed at Brazilian National Cancer Institute (INCA), Rio de Janeiro, Brazil, with more than one SARS-CoV-2 nasopharyngeal swab RT-PCR-positive test from April to August 2020 were included. Viral
RNA was isolated, cDNA was synthesized and complete genome amplification using ARTIC network V3 multiplex primers was done and sequenced in a MiSeq platform. Reads were assembled and consensus sequences extracted using Geneious R11. Maximum likelihood phylogenetic analysis was performed using PhyML 3.0 with representative sequences from different lineages and the ten best matched SARS-CoV-2 genomes for each timepoint consensus. Infection cases were defined according to the phylogenetic reconstruction and comparisons between the SARS-CoV-2 consensus sequences infecting the different lineages of each patient in the longitudinal analysis.

**Results:** A total of 55 samples derived from 26 different cancer patients were analyzed. Assembled genomes belong to B.1.1.33 (n=37) and B.1.1.28 (n=7) lineages. The 11 remaining were not classified due to low genome coverage. Most of the patients (n=16) showed the same identical viral sequence in the different lineages analyzed, and were classified as sustained viremia. For 4 cases, the genomes analyzed were distributed in different clades of the maximum likelihood reconstruction and were considered reinfections. Finally, six cases showed overlap virus evolution, and the timespan of the samples ranged from 7 to 78 days.

**Conclusion:** In this study, evaluating the SARS-CoV-2 complete genome from 26 cases, we report different longitudinal profiles of SARS-CoV-2 infection in cancer patients.

**SARS-CoV-2 Mild Infection as Risk Factor for Herpes Human Viruses Reactivation**

Serena Vita1, Elicia Petruccioni1, Eleonora Ciminia1, Maria Beatrice Valls1, Patrizia De Marco1, Settima Sbarra1, Stefania Notari1, Cecilia Lindestam Arlehamn1, Alessandro Sette1, Andrea Antinori1, Carla Fontana1, Fabrizio Maggi1, Delia Goletti1, Emanuele Nicastri1, for the VIROMA-INMI Group

1Lazzaro Spallanzani National Institute for Infectious Diseases, Rome, Italy. 2La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

**Background:** Reactivation of Herpes Human Viruses (HHV) has been described mostly in severe SARS-CoV-2 patients (pts) [1]. We aim to study HHV reactivation, HHV-mediated T-cell response and inflammatory milieu during SARS-CoV-2-mild infection.

**Methods:** We enrolled pts with SARS-CoV-2 infection at baseline (T0), days 7 (T7) and 30 (T30) and healthy donors (HD). We evaluated HHV-serology, HHV-DNA-plasma level and the IFN-γ production after whole blood stimulation with SARS-CoV-2-spike peptides, HHV peptides designed to elicit CD4/CD8 response for Varicella Zoster Virus (VZV), Cytomegalovirus (CMV), HHV1/2, Epstein-Barr virus (EBV), and IL-2, IL-6, IL-8, TNF-α, IFN-γ, CXCL-10 plasma levels.

**Results:** We enrolled 19 HD and 53 COVID pts with WHO median score 2 (IQR 2-4). No differences in terms of age, sex, and SARS-CoV-2 vaccinations in the 2 groups were observed. All HD referred a previous SARS-CoV-2 infection. There were no clinical HHV reactivations. At T0, no difference in lymphocytes count (while CD3 and CD4 T cells differed both with a p<0.01), in HHV-seroprevalences, HHV-antibodies level and HHV reactivation were reported between groups. Blood EBV-DNA was more commonly detected at T0-T7-T30, with no difference between groups. A higher CMV-T-cell response compared to that induced by the other HHV was constantly found in both pts (p<0.0001) and HD (p=0.003). However, the CMV-CD8 response of pts was significantly higher compared to HD at T30 (p=0.032), whereas the EBV-induced CD4 and CD8 response was lower compared to HD at T0 and T7 (T0 p=0.005; p=0.003; T7 p=0.008 p=0.003). As expected, at T7 the SARS-CoV-2 response of pts was higher compared to HD (p=0.026). In pts an increased EBV-CD8 response (T0/T7 p=0.005; T0/T30 p=0.0004) was reported. Pts showed the highest SARS-CoV-2-specific response at T7 compared to T0 (p=0.044). No difference in HD over time was found. Plasma IL-2, IL-6, IFN-γ and CXCL-10 were significantly higher at T0, T7 in pts compared to HD for all p<0.007, while no difference at T30 was observed.

**Conclusion:** Inflammatory milieu, HHV T-cells response are present during SARS-CoV-2 mild infection, despite not significant plasma HHV detection. In COVID pts we observed a consistently high CMV and a decreased EBV T-cell response, probably reflecting EBV viremia. Indeed, among HHV, the CMV response appears to be the driving force with a higher CD8-mediated proportion. The identification of subclinical HHV reactivation during SARS-CoV-2 mild infection is worthy of further investigations. The figure, table, or graphic for this abstract has been removed.
SARS-Cov-2 RNA by in situ hybridization and immunodetection visualized by confocal microscopy. Spike and serotonin were quantified in plasma.

**Results:** The frequency of CD41+ MKs in peripheral blood mononucleated cells (PBMCs) was significantly higher than healthy donors (0.28±0.05 versus 0.03±0.02) as a sign of MK infection, as we previously shown in acutely infected individuals with SARS-Cov-2 in platelets. Accordingly, in all samples analyzed, circulating MK in Long COVID sheltered both Spike and SARS-Cov-2 sRNA, but also dsRNA suggestive of viral replication. These infected MKs produced blood platelets that contain also P-Spike and SARS-Cov-2 sRNA. Platelet MKs were detected in all tested Long COVID patients. Spike protein was detected at the pg level in 30% of analyzed plasma from Long COVID but not CR individuals. The level of serotonin in platelet and of tryptophan hydroxylase-1 (TPH-1), the enzyme that regulates serotonin synthesis decreased significantly (p<0.0001) in blood of Long COVID patients compared to CR individuals.

**Conclusion:** In patients developing Long COVID, SARS-Cov-2 persists and replicates in MKs producing virus-containing platelets. The presence of spike in plasma might be an additional sign of viral persistence that could be used as a Long COVID biomarker. The presence of the virus could lead to abnormal platelet activation and the formation of microclots, which would contribute to the various symptoms and to deregulation of serotonin uptake, contributing to the neurocognitive symptoms observed in long-onset COVID.

### 349 Ultra-Low Level HIV p24 Production as a Driver of Immune Activation in Individuals Treated with ART


**University of Bonn, Bonn, Germany, 2University of Bonn, Bonn, Germany, 3University Hospital Essen, Essen, Germany**

**Background:** Similar to the persistent presence of HIV, residual immune activation has been observed despite antiretroviral therapy (ART). It was shown that in treated people with HIV, T cells remain activated even when the virus is undetectable. The causes of this chronic inflammation and immune activation in treated HIV are not fully understood.

**Methods:** Here, we have developed an ultrasensitive p24 single-molecule array to detect p24 in plasma at a 1000-fold lower concentration (fg/ml) compared to previous assays. This advancement allowed us to analyze people with chronic HIV who are undergoing treatment (with undetectable viral load) and compared it to immune correlates measured by a multicolor immune assay.

**Results:** We first investigated whether plasma p24 is detectable at ultra-low concentrations in a cohort of 172 individuals with chronic and ART-treated HIV infection, who have maintained HIV RNA levels below the detection limit (<50 HIV RNA copies/ml) for >4 years. Ultra-low level HIV p24 [range 4.5 fg/ml – 330 fg/ml] was detectable in 48 out of 172 individuals (28%). There was no significant correlation between age, gender, ART regimen and the average duration of therapy was identical between groups with or without detectable HIV p24 (p24+: 8.4 years [4.3 – 17]; p24-: 8.4 years [4 – 30]; p>0.05). Next, we hypothesized that ongoing p24 production is responsible for low level immune activation. Indeed, CD8 and CD4 T cells from individuals with detectable HIV p24 showed significantly increased expression of the activation marker CD38 compared to individuals without detectable p24 (p < 0.05). Interestingly, individuals with detectable HIV p24 had significantly higher HIV-specific CD8 T cell responses (p<0.05) despite ongoing treatment, indicating that they are still able to recognize HIV infected cells. We also determined HIV p24 concentration behavior before and after ART initiation as well as from acute to chronic infection. Therefore, we studied 43 people who initiated suppressive ART during acute HIV and remained virally suppressed over a 2-year period. Despite HIV-1 RNA that declined to undetectable levels (<30 copies/ml), p24 levels remained detectable in 25% of individuals one year and 19% of individuals two years after ART start.

**Conclusion:** Despite continuous ART, we were able to detect ultra-low level of p24 production, suggesting either ongoing viral replication or active transcriptional HIV integration sites can be the primary driver of HIV immune activation.

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350  CAR86 Activation in Myeloid Cells as a Driver of Inflammation in HIV Infection
Marilla R. Pinzone, Liang Shan
Washington University at St Louis, St Louis, MO, USA

Background: Myeloid cells play an essential role in HIV persistence and chronic inflammation. Inflammation activation in response to "danger-associated stimuli" can lead to pyroptotic cell death and release of inflammatory cytokines such as IL-1β and IL-18. HIV-1 protease has been shown to activate the CAR86 inflammasome in CD4 T cells, which undergo pyroptosis but do not produce IL-1β and IL-18. We hypothesize that CAR86 can also be activated in myeloid cells upon exposure to HIV, which are poised to drive inflammation.

Methods: Monocyte-derived macrophages (MDMs) were cocultured with HIV-infected autologous CD4 T cells. In selected experiments, CAR86 knockout (KO) cells were used for coculture. Supernatants were collected at 8 hours for IL-1β and IL-18 ELISA. P values were calculated using t test or ANOVA test.

Results: Coculture of MDMs with autologous CD4 T cells infected with CCR5-tropic HIV isolates resulted in increased release of IL-1β compared to uninfected cocultures (n=4, 128 vs 5 pg/ml, p=0.02) and MDM alone (128 vs 3.8 pg/ml, p=0.03) at 8 hours. Similar increases were observed for IL-18. Coculture with CD4 T cells infected with X4-tropic HIV did not increase IL-1β or IL-18 release. The release of inflammatory cytokines was prevented by pre-incubation with the caspase-1 inhibitor VX-765 or maraviroc. Coculture using CAR86 KO cells resulted in significantly lower IL-1β (43 vs 292 pg/ml, p=0.03) and IL-18 (224 vs 1384 pg/ml, p=0.0005) levels compared to Cas9 only controls.

Conclusion: Activation of the CAR86 inflammasome upon coculture with infected CD4 T cells results in release of inflammatory cytokines from autologous myeloid cells. This is abolished by CAR86 knockout as well as by use of drugs that either prevent HIV entry in myeloid cells or block the downstream signaling through caspase-1. Our findings offer novel clues into HIV pathogenesis by providing a mechanism for myeloid cell activation observed during untreated infection. Since some HIV proviruses remain translationally active even during effective antiretroviral therapy, it is possible that HIV proteins can continue to fuel inflammation by activating CAR86 in myeloid cells even when new rounds of infection are prevented. Therefore, it is important to understand whether CAR86 inhibition could decrease chronic inflammation in people living with HIV.

352  Immunosuppressive Effects of LLDT-8 in ART-Treated SIV-Infected Rhesus Macaques
Xiaosheng Liu1, Tingxia Lv1, Jing Xue1, Ling Lin1, Lafeng Lu1, Xiaodi Li4, Yang Yang3, Yuanni Wu1, Qiang Wei2, Wei Cao4, Taisheng Li4
1Sunhwa University, Beijing, China; 2Beijing Friendship Hospital, Beijing, China; 3Chinese Academy of Medical Sciences, Beijing, China; 4Peking Union Medical College Hospital, Beijing, China

Background: Chronic immune activation plays a significant role in the pathogenesis and disease progression of human immunodeficiency virus (HIV), and the existing interventions to address this issue are limited. In a phase II clinical trial, (SRU)–5–hydroxytryptolide (LLDT-8) demonstrated promising potential in enhancing CD4+ T cell recovery. However, the precise mechanism of action of LLDT-8 remains to be explored.

Methods: To assess the treatment effects of LLDT-8, we conducted flow cytometry and RNA-seq analyses on eight Chinese rhesus monkeys infected with simian immunodeficiency virus (SIV). Additionally, we performed comprehensive transcriptional analyses, including cross-sectional and longitudinal analysis. The cross-sectional and longitudinal analysis identified 2531 and 1809 DEGs, respectively. GSEA analysis indicated that LLDT-8 treatment led to significant downregulation of proliferation-related pathways, such as E2F targets, G2M checkpoint, and mitotic spindle pathways. WGCNA analysis identified two modules and 202 hub genes associated with CD8 activation levels. Deconvolution analysis showed a significant decrease in the proportion of CD8+ T cells and activated CD4+ T cells during LLDT-8 treatment. Gene ontology results demonstrated that the common DEGs between LLDT-8-treated patients and rhesus monkeys were primarily enriched in cell activation and cell cycle progression. Furthermore, in vitro cellular experiments validated the consistent impact of LLDT-8 in inhibiting proliferation, activation (HLA-DR and CD38 expression), exhaustion (PD-1 expression), and IFN-γ production in human CD4+ and CD8+ T cells.

Conclusion: LLDT-8 exhibited notable efficacy in alleviating immune activation in both an in vivo animal model and in vitro human cell experiments. These findings suggest that LLDT-8 may hold potential as a drug for managing systemic immune activation associated with SIV/HIV infection, warranting further prospective clinical explorations.

353  Circulating Immunoregulatory Proteins Indicative of Poor CD4 Recovery in People With HIV on ART
Preeti Moar1, Thomas A. Premenza1, Scott Bowler2, Courtney Fрайer2, Sara Gianella3, Alan Landay3, Lishomwa Ndhlovu1
1Weill Cornell Medicine, New York, NY, USA; 2University of California San Diego, La Jolla, CA, USA; 3Baylor University, Collin, TX, USA

Background: One-third of people with HIV (PWH) demonstrate poor CD4+ lymphocyte recovery (<200 cells/ml) despite suppressive anti-retroviral therapy (ART). Poor CD4 recovery is associated with persistent immune activation and inflammation, severe immune dysfunction, adverse comorbid outcomes, and mortality. Soluble immunoregulatory proteins and lymphocyte receptor/ligands that function in activation or inhibition are elevated in PWH, and associated with HIV-specific T cell function, HIV reservoir size, and comorbid outcomes during ART. The potential mechanism of poor immune reconstitution and relationship of poor CD4 recovery with lymphocyte-associated immunoregulatory proteins remains unclear. Here we assessed a panel of circulating immunoregulatory proteins and their association with poor CD4 recovery.

Methods: Study participants enrolled in the AIDS Clinical Trials Group Longitudinal Linked Randomized Trials (ALLRT) cohort were stratified in poor CD4 recovery (CD4 <200, n=34) or CD4 count normalization (CD4 >500, n=82)
Suppressing Asymptomatic CMV With Letermovir Reshapes Immunoregulatory Viral IL-10

Background: While human IL-10 levels were unchanged. While sTNFR2 increased, 30 plasma
consistent with this hypothesis, plasma IL-10RA – a marker of IL-10 receptor
immunoregulatory viral IL-10 may have caused the increase in plasma sTNFR2.

Methods: Of 42 participants enrolled, 40 contributed to the week 8 per-protocol
analysis, stratified by CD4 count (45% <350 cells/mm³). Two separate biomarkers (ELISA and Olink Inflammation and Cardiometabolic Explore panels)
were measured in treated HIV by Mendelian Randomization (AXL, GAS6, and IL-6R; nominal
P≤0.05). Conversely, we observed a significant increase in plasma Gal-9, CD276, and OX40 among participants with poor CD4 recovery as compared to those with normalized CD4 count (all P<0.05) [Figure 1A]. Gal-9, CD276, and OX40 remained significant in logistic regressions adjusted for age (all P<0.05). Further, a boosted decision tree model consisting of age and the six differential immunoregulatory proteins accurately classified individuals with poor CD4 recovery from those with reconstituted CD4 counts (AUC =0.902 +/- 0.078) [Figure 1B]. In this model, Gal-9, CD276 and Gal-9 had the highest feature importance [Figure 1B].

Conclusion: We found a novel signature of circulating lymphocyte-associated immunoregulatory proteins indicative of poor CD4 recovery and potential targets to monitor immune perturbations in PWH during suppressive ART.
356 Methamphetamine Use in PWH on ART Is Associated With Inflammation and Residual HIV Transcription
Maria Sophia B. Donaire1, Fernanda C. Coirada2, Sun Jin Kim3, Sannidhi Sarvadhavabhatla, Vivian Pae4, Alton Barbehenn4, Cassandra Yunn5, John C. Halifax2, Anna Rull1, Marina Flores-Piñas4, Alexy Inciarte4, Virginie Sheikh5, Irini Sereti2, Adam Rupert7, Cassandra Yun1, Jing Wang2, Sannidhi1, Vivian Pae2, John C. Halifax2, Steven A. Yukl2, Nittaya Phanuphak1, Frederick K. Sawe1, Jintanat Ananworanich1, Silvia Chafino1, Fernanda C. Coirada4, Sulggi A. Lee1

1University of California San Francisco, San Francisco, CA, USA, 2Tspyer University, Atlanta, GA, USA

Background: High-risk people with HIV (PWH) such as individuals who use methamphetamine (MA) are most likely to benefit from HIV eradication strategies and yet they often have high rates of suboptimal ART adherence. No study to date has evaluated whether PWH with adequate viral suppression and who use MA have elevated levels of systemic inflammation and residual viral transcription during ART, which may pose additional challenges to HIV cure in this population.

Methods: We performed a pilot study of 20 PWH with and without MA use (10 HIV+MA+, 10 HIV+MA-). Inclusion criteria were confirmed HIV-1 infection and undetectable viral load (<40 copies/ml) for at least 1 year. HIV+MA+ participants were sampled at 2 timepoints, and MA concentrations were quantified from plasma using a clinically validated liquid-chromatography tandem mass spectrometry (LC-MS/MS) assay. Plasma samples were also used to quantify 43 analytes using a multi-plex chemiluminescence immunoassay (Mesoscale Discovery). PBMCs were used to perform reverse transcription droplet digital PCR (RT-ddPCR) assays to quantify HIV RNA transcripts produced during sequential stages of viral transcription reflecting transcriptional initiation (TAR), elongation (Long LTR), mid-transcription (Pol), distal transcription (Nef), completion (PolyA), and multiple splicing (Tat-Rev) events. Wilcoxon rank sum and signed rank tests, as well as linear regression models, were used to perform across- and within-individual comparisons.

Results: HIV+MA+ and HIV+MA- groups were balanced by age, gender, race/ethnicity, nadir CD4+ T-cell count, and duration of ART. Among the 43 analytes, only TNF-α, TNF-β, IL-6, and MIP-1α were significantly higher in HIV+MA+ vs. HIV+MA- individuals, and these associations (except IFN-β) remained statistically significant in multivariate models adjusted for nadir CD4+ T-cell count and duration of ART (P<0.05). HIV RNA transcripts were detectable in a total of 17 participants. HIV Pol transcripts were significantly higher in 8 HIV+MA+ vs. 9 HIV+MA- participants.

Conclusion: To our knowledge, this small pilot study is the first human study to evaluate the impact of MA use on circulating cytokine levels and the HIV reservoir during suppressive ART. MA has been shown in animal and in vitro studies to increase T cell activation and exhaustion and enhance HIV transcription. Our findings suggest that even during ART suppression, PWH who use MA may have higher levels of systemic inflammation and residual HIV transcription.

Figure 1. Methamphetamine use in people with HIV on ART was associated with increased plasma markers of inflammation (A) and residual viral replication (B).

357 Singular CD4+ and CD8+ T-Cell Proteomic Profiles in PLHIV Immunological Non-Responders Before ART
Marina Flores-Piñas1, Silvia Chafino, Consuelo Viñals, Pere Domingo, Miguel López-Dupla, Alexey Inciarte, Jordi Navarro, Julio Blasco, Francesc Vidal, Joaquim Pareigis, Anna Bull

1Hospital Universitario de Tarragona Joan XXIII, Tarragona, Spain, 2Sant Pau Biomedical Research Institute, Barcelona, Spain, 3Hospital Clinic of Barcelona, Barcelona, Spain, 4VirVax Heron Research Institute, Barcelona, Spain, 5ViroCay Instituto for AIDS Research, Badalona, Spain, 6Rovira i Virgili University, Tarragona, Spain.

Background: A significant proportion of people living with HIV (PLHIV) who achieve virological suppression with antiretroviral therapy (ART) fail to recover CD4+ T-cell counts, these patients are known as immunological non-responders (INR). Multiple complex mechanisms are involved in the failure of immune recovery, and intracellular proteins of CD4+ and CD8+ T-cells may have an important role. The main aim is to identify proteins, or a group of proteins, that...
by sex. These findings suggest that there may be distinct pathophysiological mechanisms that account for the observed increased risk of death seen in female PWH that warrant further investigation.

**Glycomic Markers of Biological Aging in Trans Women With HIV Versus Cis Men and Cis Women With HIV**

Leila B. Giron, Ana N. Hyatt, Mohamed Elkaid, Paula Debroy, David B. Hanna, Igbo Ofoekon, Margaret A. Fischl, Daniel Merenstein, Sabina Haberlen, Alan Landay, Frank Palella, Phyllis Tien, Todd T. Brown, Jordan E. Lake, Mohamad Abdel-Mohsen

**Methods:** Plasma samples were collected from 22 transgender women with HIV (TWWH); all were on estrogen, 50% were on spironolactone/antiandrogen, and 32% having testosterone levels <50ng/dL. These samples were age (± 5 years), race/ethnicity, and body mass index category matched with samples from 20 cisgender men with HIV (CMWH) and 18 cisgender women with HIV (CWWH; 6 pre-menopausal and 12 post-menopausal). All participants were on ART and had viral load <50 copies/ml. We measured two categories of plasma-based markers of aging: 1) 20 markers of inflammatory aging (based on PMID: 34888528) using multiplex cytokine arrays, and 2) 31 IgG glycomic markers of biological aging (based on PMID: 24325898) using capillary electrophoresis. Kruskal-Wallis tests and Spearman correlations were used for analyses, and false discovery rates (FDR) were calculated to correct for multiple comparisons.

**Results:** While we observed no differences in levels of inflammation markers between TWWH and CMWH, there were significant differences in levels of several IgG glycomic markers of aging between the two groups. Notably, levels of several galactosylated glycan, which are linked to younger chronological and biological age, were higher in TWWH compared to CMWH (FDR<0.05; Fig. 1A). Conversely, several agalactosylated glycan, linked to older age, were lower in TWWH compared to CMWH (FDR<0.05; Fig. 1B). These effects were consistent among TWWH, regardless of whether they had testosterone suppression or not. The anti-aging glycomic profiles of TWWH resembled those of pre-menopausal CWWH, while the pro-aging profiles of CWWH resembled those of post-menopausal CWWH. The anti-aging profile, enriched in TWWH, was correlated with lower levels of several inflammation markers, including CXCL1, TNF-α, and IP10, while the pro-aging profile, lower in TWWH, was associated with higher inflammation, especially in TWWH.

**Conclusion:** TWWH exhibit anti-aging glycomic profiles that resemble those of pre-menopausal CWWH. Longitudinal studies are needed to better understand the relationships between GAHT, biological aging, and the risk of developing age-associated diseases. Mechanistic investigation is also warranted to explore the potential impact of GAHT and sex hormones on aging processes.

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360 Signature of CD4 T Cells During Acute HIV-1 Infection Is Predictive of Disease Progression

Dominic Paquin-Proulx, Bonnie M. Sliker, Ningbo Jian, Boonrat Tassaneenithipat, Leigh-Anne Eller, Gina Donofrio, Matthew Creggan, Sandhya Vasan, Julie Ake, Mary A. Marovich, Nelson L. Michael, Merlin L. Robinb, Michael A. Eller, Shelly J. Krebs, for the RV+ Study Team

**Background:** Immune activation, a hallmark of chronic HIV infection, is a major contributor of HIV pathogenesis, even in the context of viral suppression by antiretroviral therapy. The magnitude of immune activation during HIV infection is both established early and is more predictive of the rate of disease progression than plasma viral load. Elucidating the critical events in acute HIV infection (AHI) associated with disease progression will facilitate the design of new approaches to achieve HIV remission.

**Methods:** To investigate the dynamics of inflammation and immune activation during AHI, we utilized samples from 17 Thai and 26 East African participants in the RV217 AHI study. We measured the levels of 26 soluble markers of inflammation inclusive of chemokines, cytokines, and markers of microbial translocation starting from pre-infection through AHI. We determined the dynamics of cellular immune activation by assessing CD4 and CD8 T cell activation during AHI using flow cytometry. The level and frequency of immune activation was used to predict a CD4 T cell count <350 using nonparametric rank-based testing, logistic regression, and cumulative incidence. The prediction capability of identified markers was also examined by Area under the Receiver Operating Characteristic (AUCROC) curve. The level of soluble and cellular markers of immune activation between East Africa and Thailand significantly differed between regions both before and during AHI. Similarly, variable signatures of immune activation were associated with peak and set point viral loads for each region. IL-10, IL-8 and IL-10 levels were associated with peak viral load in Africa but not in Thailand. Levels of IL-1P pre-infection in Thailand, but not in East Africa, were inversely associated with peak and set point viral loads. Combining datasets, higher levels of CCR5 and CD38 expression on CD4 T cells at 4.5 weeks post 1st HIV RNA positive test (around set point viral load) were predictive of reaching a CD4 count <350 independently of region or viral load.

**Conclusion:** Our results show that the frequency of CD4 T cells expressing CCR5 and CD38 at set point viral load is associated with faster disease progression independent of viral load. This suggests that CD4 T cells circulating with an activated phenotype associated with susceptibility to viral entry may be predictive of immune pathology.

361 Distinct Plasma Protein Changes Precede Loss of Spontaneous HIV Control

Nadira Vadaq, Albert L. Groenendijk, Jessica D. Santos, Wilhelm A. Vos, Marc Blaauw, Louise E. van Eerkerken, Leo Josten, Vasiliki Matzaraki, Jan van Lunzen, Susan M. Schader, Casper Rosk, Annelles Verbon, Mihai Netea, Ferdinand Wilt, Andre J. van der Ven

**Background:** Understanding antiretroviral therapy (ART)-independent control of HIV is of paramount importance and central for HIV cure. Some people living with HIV spontaneous regulate viral replication without ART and are categorized into ‘elite controllers’ (EC, HIV- RNA <50 c/ml) and ‘viremic controllers’ (VC, HIV- RNA between 50-10,000 c/ml). EC or VC may eventually lose controller status (HIV-RNA >10,000 copies/ml) and become transient controllers (TC), in contrast to persistent controllers (PC). Exploring plasma protein profiles of controllers that transition to TC may discern the intricacies of HIV pathogenesis and may ultimately yield new treatment strategies. Our aim was to scrutinize
whether circulating proteins of EC and VC at baseline were associated with loss and/or sustained viral control.

Methods: The Dutch national ATHENA cohort provided baseline blood samples from 36 ECs and 145 VC s, maintaining control status for >5 years. Serial viral load (VL) measurements were documented for up to 17 years. Expression of 3072 plasma proteins was measured using proximity extension assay coupled with next-generation sequencing, with 2420 proteins used for analysis after quality control. Only one EC lost control during follow-up; analysis was therefore applied on VC only.

Results: Loss of controller status occurred in 38% (55/145) of VC s after a median of 8.5 years. TC had similar demographics (age, sex, BM I, ethnicity), smoking status, and latest CD4 and VL compared to PC s. However, elevated initial VL was noted in TC s (median Interquartile range) TC s 1347 [245, 4271] vs. PC s 443 [50, 1912.5] (c/ml). Over time, TC exhibited an annual increase in VL by 1375 c/ml, while PC s exhibited stable VL. Proteomic analysis identified seven proteins (ZBP1, SH2D1A, CDH15, GIMAP7, VPS6C10, NDRG1, GZMH) associated with a higher risk of losing VC status, whereas CNGB3 was linked to a lower risk, observed over a median period of 4.2 years before loss of HIV control. Notably, certain proteins (SH2D1A, NDRG1) have been implicated in other viral infections, yet their association with HIV infections is scarce or absent in existing literature.

Conclusion: Several years before loss of spontaneous viral control, TC exhibited the upregulation of plasma proteins with known immune and cellular functions, including inflammation, apoptosis, and cell adhesion. Our findings advance our understanding of ART-independent control mechanisms, underscoring the prospect of identifying early biomarkers for impending loss of HIV control.

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**362 HIV-Induced Lung Inflammation in Humanized Mice**

Leyao Wang, Sara Nicholson, Hongbo Guo, Liang Shan
Washington University in St Louis, St Louis, MO, USA

**Background:** HIV infection-related lung diseases continue to be one of the leading causes of morbidity and mortality for people living with HIV. The cross-sectional design of population studies has made it impossible to elucidate a causal relationship between immune biomarkers and lung disease. Therefore, we aim to establish an animal model to longitudinally monitor HIV infection and lung immune activation and perturbation.

Methods: We established HIV infection in a humanized mouse model named MISTRG-6-15, in which several human cytokine-coding genes, including M-CSF, IL-3/GM-CSF, SIRPa, TPO, IL-6, and IL-15, were knocked into their respective mouse loci to promote human hematopoietic stem and progenitor cells (HSPCs) for self-renewal and differentiation. We isolated human CD45+ cells from the lungs of HIV-infected and control mice and performed single-cell RNA sequencing and ex vivo functional analyses.

Results: MISTRG-6-15 mice engrafted with cord blood HSPCs developed human myeloid cells (monocytes, macrophages, and neutrophils), T cells, B cells, NK cells, and other innate lymphoid cells (ILCs) in the lungs. The HIV-infected MISTRG-6-15 mice had a systematic augment of interferon gamma and alpha pathways across all cell types in the blood, spleen, and lungs. We found significant depletions of lung effector and tissue-resident CD4+ T cells and CD4+ regulatory T cells, as well as many subsets of antigen presenting cells (APCs). By contrast, CD8+ T cells, NK cells, and ILCs expanded and produced inflammatory cytokines in the infected lungs, which was in line with human studies. Genes associated with cell proliferation and interferon responses were significantly upregulated in the lungs of HIV-infected mice. Compared to the blood and spleen, the lungs had a larger amount of differentially expressed genes between the infected and un-infected mice. Further subset analyses identified that T-cells (both CD4+ and CD8+ T-cells) and myeloid cells specifically had significantly more differential expressed genes in the lungs than in the blood and spleen.

Conclusion: Our results demonstrated that MISTRG-6-15 mouse can be a useful model to evaluate lung inflammation driven by HIV infection. We identified unique features of HIV-induced immune perturbation in the lung. This humanized mouse model allows us to perform mechanical studies to better understand HIV-driven lung complications.

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**363 Exogenous Estrogen Increases HIV Target Cell Frequency in the Rectal Mucosa of Male Primates**

Patricia A. Hahn, Eric S. Alexander, Kimberly Weissgrau, Tianling Ou, Wenhui He, Eva Rakasz, Michael Farzan, Joseph R. Kurian, Mauricio A. Martins

1The Herbert Wertheim OF Scripps Institute for Biomedical Innovation & Technology, Jupiter, FL, USA,
2Wisconsin National Primate Research Center, Madison, WI, USA,
3Boston Children’s Hospital, Boston, MA, USA

**Background:** Transgender women (TGW) are 49-66 times more likely to be infected with HIV than individuals over age 15. Considering TGW’s high risk of contracting HIV, they stand to benefit greatly from anti-HIV therapeutics, but little is known about the immunomodulatory effects of feminizing hormone therapy (FHT). To relieve gender dysphoria and facilitate physical feminization, many TGW utilize FHT, consisting primarily of 17β-estradiol (E2), which has immune-enhancing effects. It is well established that estrogen can influence T-cell development and impart higher resistance to infection in women versus men. Since estrogen can also amplify antibody responses, FHT could have both positive and negative immune consequences to TGW. Hence, to advance our understanding of the immunomodulatory effects of FHT in TGW, we set out to model FHT in rhesus macaques.

Methods: Using slow-release subcutaneous E2 pellets, we developed a dosing regimen in male rhesus macaques that elevate levels of E2 and suppressed testosterone. We used flow cytometry to measure cellular dynamics and ELISA to evaluate the magnitude of LNP/mRNA vaccine-induced Env-binding IgG antibodies.

Results: The E2 regimen significantly increased serum E2 concentrations and suppressed endogenous testosterone levels, while also inducing physical traits associated with feminization, like enlarged nipples. Importantly, immunophenotyping analysis revealed that CCR5+ CD4+ T-cells, the primary targets of HIV infection, were significantly elevated in both blood and gut from the E2-treated animals. Although the female sex is associated with enhanced immune responses to vaccines, FHT did not significantly affect vaccine-induced anti-Env antibodies in the E2 group following mRNA vaccination.

Conclusion: These results demonstrate for the first time the feasibility of modeling gender-affirming hormone therapy in rhesus macaques and implicates FHT as a potential driver of HIV susceptibility in TGW.

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**364 Enema Use Minimally Impacts Immunity and Does Not Affect Susceptibility to Low-Dose Intrarectal SIV**

Alexandra Ortiz, Fabiola Castello Casta, Brandon Keele, Jason Brenchley

1National Institutes of Health, Bethesda, MD, USA, 2AIDS and Cancer Virus Program, Fredenick, MD, USA

**Background:** Men who have sex with men (MSM) are disproportionately affected by HIV. Increased susceptibility to HIV in MSM is attributed to high frequencies of susceptible CD4+ T-cells, GI epithelial barrier disruption, and dysbiosis of the intestinal microbiome. Although colorectal epithelial
barrier damage is due, in part, to inflammation resulting from receptive anal intercourse, rectal douching has also been proposed as a significant contributor. The effects of repeated enema use on the composition of the GI tract microbiome, intestinal immunity, and susceptibility to rectal HIV acquisition have not been empirically assessed.

Methods: We administered enemas (Normosol-R) to rhesus macaques (Control/Enema, n=6/6) thrice-weekly, prior to repeated low-dose intra-rectal SIVmac239X challenge. Before and 28 days after enema treatment initiation (n=12 enemas) we assessed the fecal microbiome by 16S Illumina sequencing and surveyed intestinal and systemic immunity by flow cytometry, multiplex immunologic transcript quantification (NanoString), and ELISA. Fifty-six days after enema initiation (n=24 enemas) we challenged animals with 4 TCID_50 SIVmac239X until infection was confirmed. Susceptibility to SIV infection in our animals was assessed both by time to SIV infection and number of acquired transmitter-founder (T/F) variants.

Results: Prior to SIV challenge, we observed that as compared to control animals, repeated enema administration was associated with fewer memory CD4+ T-cells in the jejunum (p<0.001) and fewer memory CD4+ (p=0.032) and CD8+ (p=0.016) T-cells in the peripheral blood of treated animals. Treatment was also associated with a trend for less IL-22 production from intestinal memory CD4+ T-cells. No post-treatment differences were observed in plasma sCD14 or iFABP2. Few differences were observed in the composition of the fecal microbiome, with enema treated animals displaying perturbations in the representation of some Oscillosporales species. Of animals infected thus far (n=3/6 each group), there are no differences in the number of challenges that resulted in successful infection nor in the number of acquired T/F variants.

Conclusion: Our analyses will provide a detailed assessment of how the microbiome and intestinal immunity change in response to repeated enema usage. Insight gained from our comprehensive study will inform the causes and consequences of repeated enema usage in MSM and will inform the design of improved bowel-cleaning preparations for sexual and surgical use.

SIV Infection in Sooty Mangabeys Does Not Impact Survival but Changes Cause of Death

Cristina Ceriani, Brianne Beetsner, Maria Crane, Joyce Cohen, Ian N. Moore, Deanna A. Kulpa, Guido Silvestri
Emory University, Atlanta, GA, USA

Background: Sooty mangabeys (SMs) are natural host of simian immunodeficiency virus (SIV), and do not progress to AIDS despite high viral replication. The main factors involved in the benign nature of this infection are (i) low level of immune activation, (ii) relative preservation of specific CD4+ T-cell subsets from direct virus infection, and (iii) absence of microbial translocation from the gut to the systemic circulation. Extensive documentation supports the non-pathogenic nature of SIV infection in SMs, with no significant disparities observed between SIV-infected and uninfected SMs.

Methods: To better assess the long-term impact of SIV infection on the overall clinical conditions of SMs, we have conducted a systematic analysis of the causes of death in 307 SMs, of which 219 SIV-infected and 88 uninfected. We were housed at ENPRC and had died of natural (i.e., non-experimental) causes between 1986 and 2022.

Results: We found that SIV-infected SMs live ~4 years longer than SIV-uninfected SMs, although this result is hard to interpret due to differences in the way animals were housed and included in specific experimental studies. While the causes of death were not different between SIV-infected and uninfected SMs that died before age 15, we found a significant differences in the relative frequency of specific causes of death in the geriatric population (>15 y.o.). Specifically, we observed that SIV-infected SMs were more likely to die from infections (Odds Ratio (OR) + infinity, 95% CI 2.07- + infinity, OR = 0.0064) but less likely to die from cardiovascular disease (OR 0.232, 95% CI 0.104-0.675, P = 0.00613) as compared to uninfected animals. A similar trend was consistently observed within the subgroups categorized by sex, indicating that these findings were robust across sexes. No differences were observed for cancer, diabetes, trauma, and miscellaneous other causes.

Conclusion: While confirming the non-pathogenic nature of SIV infection in SMs, these data reveal, for the first time, a qualitative impact of SIV infection on the host physiology that induces a significant change in the pattern of mortality in these natural SIV hosts.

Positive Reinforcement Training Reduces Stress in SIV-Infected Macaque Models of HIV Infection

Selena M. Guerrero-Martín, Bless W. Carlsson, Samuel A. Brill, Erin N. Shink, Suzanne Queen, Leah H. Robin, Melanie J. Graham, Lydia M. Hopper, Lucio Gama, Christine Zink, Joseph Mankovskis, James E. Clements, Kelly A. Metcalf Pate
The Johns Hopkins University School of Medicine, Baltimore, MD, USA, University of Minnesota, Minneapolis, MN, USA

Background: Work with SIV infected macaque models is essential to HIV pathogenesis, vaccine development and cure research. Positive reinforcement training (PRT) may be used rather than restraint or sedation to facilitate administration injectable antiretroviral therapy (ART) or obtain blood samples. The effect of PRT on data in these models has yet to be defined.

Methods: A subset of juvenile male rhesus (Macaca mulatta) and pigtailed (Macaca nemestrina) macaques were trained using PRT to voluntarily present a limb for ART injection and blood sampling prior to intravenous infection with SIVmac251 or SIV17E/Fr combined with SIV delta B670, respectively, and compared to untrained SIV infected macaques, and subsequently treated with ART to suppress viral replication. Regardless of training history, macaques were sedated throughout infection to obtain blood and cerebral spinal fluid. Viral loads, immune markers, and plasma cortisol levels were compared between trained and untrained SIV infected macaques (M. mulatta; n = 24 trained; n = 8 untrained; M. nemestrina n = 6 trained; n = 29 untrained) throughout the course of infection using a mixed effects model.

Results: No differences were observed between trained and untrained M. mulatta in peripheral or central nervous system (CNS) viral loads during acute infection, though trained M. nemestrina demonstrated significantly lower viral loads compared to untrained macaques; no differences in viral suppression were observed upon ART initiation in either species. CD4 T cell numbers declined in all animals during acute SIV infection, with trained rhesus macaques demonstrating a delay to decline compared to untrained animals. Though all macaques experienced increased plasma cortisol following SIV infection, trained macaques had a lesser increase compared to untrained animals.

Conclusion: PRT buffers the physiologic stress associated with SIV infection in macaques, as evidenced by a less pronounced cortisol response to infection. Further work needs to be completed to understand the extent to which PRT may affect viral load and immune parameters, and caution should be exercised when comparing data from animals that have engaged in PRT compared to untrained macaques.

HIV Rapid Intra-Host Evolution Allows Evasion From VRC01 Infusion via Positive Selection

Frida Belinsky, Sung Hee Ko, Pierce Radecki, Vanessa Guerra, Emily Coates, Pamela Costner, Julie Ledgerwood, John R. Mascola, Eli Boritz
National Institutes of Health, Bethesda, MD, USA

Background: One of the factors contributing to HIV’s persistence is its rapid evolution. Understanding how the env gene evolves under antibody-mediated pressure is particularly important for efforts to use antibodies as therapies for HIV. In a previous clinical trial, infusion of the broadly-neutralizing antibody (bNAb) VRC01, which targets the CD4 receptor binding site, lowered plasma HIV viremia in a subset of participants, but was associated with the emergence of neutralization-resistant env variants. Here we sought to develop an analytical approach for identifying env mutations that confer escape from antibodies using single-genome sequence data from participants treated with VRC01.

Methods: We applied single-genome amplification and sequencing (HT-SGS) using unique molecular identifiers (UMIs) and the Pacific Biosciences long-read platform, to measure HIV env allele frequency changes over time in longitudinal samples from people with chronic, untreated HIV infection who received one dose of VRC01. Eight participants were studied, and a total of 29,433 sequences were analyzed. We used two approaches to identify changes adaptive to VRC01 escape: (1) a codon-based DNUS approach executed by HYPHY with FUBAR [2] a population genetics approach where Tajima’s D was used to identify positive selective sweeps along the env gene, and then a score for the selected allele favored in evolution (SAFE) was calculated.

Results: In six of eight hosts the viral populations pre and post infusion were distinct. Specifically, changes in the VRC01 epitope were observed. In two individuals, both FUBAR and SAFE approaches identified the same positions as adaptive. In another four individuals FUBAR and SAFE pointed to different residues as adaptive. Examining manually regions of selective sweeps as identified by negative values of Tajima’s D, revealed complex evolutionary
scenarios, and suggested the existence of several adaptive mutations in each host. Furthermore, epistasis between positions is likely, due to existence of low frequency combinations, of the adaptive alleles, that do not grow in frequency. In two individuals, subpopulation structure correlating with sequence signatures of cell tropism is associated with different escape mutations.

**Conclusion:** These results suggest diverse adaptive pathways to bnAb escape among different individuals. While some mutations are easily identified as adaptive, multiple escape mutations, epistasis and tropism associated mutation are more challenging to identify and interpret.

### 368 A Rare Molecular Signature in HIV-1 Env V1 Associates With bnAb Evolution


1 University of Zurich, Zurich, Switzerland; 2 University Hospital Zurich, Zurich, Switzerland; 3 Los Alamos National Laboratory, Los Alamos, NM, USA; 4 University of Cape Town, Cape Town, South Africa; 5 University of the Witswatersrand, Johannesburg, South Africa

**Background:** Identifying traits of HIV-1 Envelope (Env) linked with broadly neutralizing antibody (bnAb) development is critical for designing bnAb vaccines. Here we report a rare twin cysteine (Cys) motif in the variable loop 1 (V1) region that is enriched among Env of bnAb inducers.

**Methods:** V1 Cys insertions were functionally characterized in vitro and quantified in Env sequence datasets from bnAb inducer cohorts (Swiss 4.5K), longitudinal cohorts (ZEPHIR, SHCS, CAPRISA) and the LANL sequence database. Factors associated with frequency of V1 Cys insertions were analyzed using logistic regression.

**Results:** Studying Env from 35 bnAb inducers from the Swiss 4.5K screen we noted a high overall neutralization resistance and observed a rare twin Cys motif in V1 in several Envs. Functional studies with bnAb inducer Env and analysis of the CATNAP database showed that Envs with the V1 Cys motif had a modestly increased neutralization resistance pointing towards a compensatory stabilizing effect. Analyzing Env sequences from 1,105 Swiss 4.5K participants, we observed an independent association with neutralization where >20% of elite neutralizers carried the motif compared to 5% of non-neutralizers. Sequence simulations and comparison to >600 Env sequences of the LANL database showed that the observed frequency of Env with two extra Cys in V1 is unlikely to occur by chance. Twin V1 Cys occur in recent transmission, suggesting no transmission bias, and show peak frequencies in later infection. Longitudinal Env profiles of 57 CAPRISA donors showed fluctuating frequencies of variants with extra V1 Cys, suggesting the motif alone provides a limited fitness advantage. Notably, a high proportion of Env with twin V1 Cys was transmitted in the AMP trial placebo arms (15% and 9% of participants in IPOWER and 704, respectively) while breakthrough viruses showed a VRC01 treatment dose dependent reduction in twin V1 Cys, suggesting a fitness deficit of transmitting twin V1 Cys viruses.

**Conclusion:** Our data support a role of the V1 Cys motif in optimizing V1 stabilization and epitope shielding during neutralization escape. Gains in neutralization resistance and viral fitness through the motif may vary depending on the extent of virus naïve co-evolution. BnAb-experienced viruses are likely optimized for both fitness and resistance, while recently escaped variants may, despite twin V1 Cys insertions, still have fitness deficits and are rapidly counter-selected when new pressure arises as in the case of VRC01 variants may, despite twin V1 Cys insertions, still have fitness deficits and are depending on the extent of virus nAb co-evolution. BnAb-experienced viruses in neutralization resistance and viral fitness through the motif may vary stabilizing effect. Analyzing Env sequences from 1,105 Swiss 4.5K participants, we observed evidence of strong viral selection by post-SHIV week 16 at the N terminus of the fusion peptide and at the structural adjacent regions, including residues nearby glycan 88. Molecular dissection of these responses into component antibody specificities by antibody isolation and cryo-EM structure determination revealed 15 of 16 isolated antibodies with cross-clade neutralization breadth to be directed towards the fusion peptide-site of vulnerability. In each macaque, isolated monoclonal antibodies recapitulated the plasma-neutralizing response (r = 0.71-0.97), with fusion peptide-binding antibodies reaching breadths of 40-60% (IC50 <50µg/ml) on a 208 strains panel. Longitudinal phylogenetic analysis revealed each of the top macaques to have only 1-2 broadly neutralizing fusion peptide-binding lineages, each induced before SHIV infection.

**Conclusion:** These results provide explicit in-vivo molecular examples for one or few B-cell lineages affording potent cross-reactive plasma-neutralizing responses. While increased titers in the current study resulted from SHIV-infection boosting, it will be interesting to test fusion peptide-Env trimer immunogens not only with altered boosting regimens, but also with altered priming regimens, such as with the escalating prime, extended-boost regimen.

### 370 Maturation Pathway of Rhesus V3-Glycan Broadly Neutralizing Antibody Lineage

**Mitchell Martin**, Tyler Evangelous, Madison Berry, Bhavna Horca, Chuanjiang Jiang, Katayoun Miansouri, Robert J. Edwards, Hai Li, George M. Shaw, Priyamvada Acharya, Kevin G. Saunders, Kevin Wiehe, Barton F. Haynes, Wilton Williams

1 Duke Human Vaccine Institute, Durham, NC, USA; 2 University of Pennsylvania, Philadelphia, PA, USA

**Background:** We previously isolated clonally-related HIV-1 envelope (Env)-reactive broadly neutralizing antibodies (bnAbs), targeted DIII030, from a pathogenic SHIV-infected rhesus macaque (RM080N021). Rhesus DIII030 targeted the V3-glycan bnAb-epitope on HIV-1 Env using an arginine (R) residue acquired via an improbable mutation from glycine (G) in the HCDR2. Engineering HCDR2-G56R mutation into the DIII030 unmutated common ancestor (UCA) alone was insufficient to achieve neutralization activity. We hypothesized that DIII030 lineage maturation included a series of mutations that preceded G56R in the HCDR2. Defining mutations associated with maturation of DIII030 will inform prime-boosting strategies to select for key mutations that confer maturation to bnAb status.

**Methods:** We isolated 62 clonally-related DIII030 Abs from RM080N021 via Env-reactive B cell flow cytometry sorting and 10X Genomics single cell immune profiling assays. DIII030 Abs clustered into three phylogenetic clades. The majority of clade 1 Abs had HCDR2-G56; all clade 2 Abs had HCDR2-G56; and all clade 3 Abs had HCDR2-R56. Monoclonal (m) Abs were tested for binding specificities, structure, and function. We expressed 26 mAbs representative of all three clades, including the inferred intermediates and UCA.

**Results:** Of 8 mAbs tested from clade 1, two containing HCDR2-G56 neutralized only autologous HIV-1 strains, but two that contained HCDR2-R56 did not neutralize HIV-1 strains tested. None of six mAbs tested from clade 2 neutralized HIV-1. In contrast to results from clades 1 and 2, all 10 mAbs tested from clade 3 with HCDR2-R56 neutralized CHB848 10.17D7 and up to 5/9 heterologous tier 2 HIV-1 from the global panel. Sequence analysis of DIII030 Abs along the path to clade 3 bnAbs revealed mutations in the HCDRs that preceded G56R. All the major clade 3 bnAbs had serine-to-proline mutation at position 114 (S114P) in the HCDR3. Mutating S114P into an intermediate Ab (IA59) along the clade 3 bnAb pathway conferred Ab binding at similar levels to the clade 3 bnAbs in contrast with wild-type IA59 which showed no binding. Computational modeling of S114P on DIII030.1-Env complex suggested favorable neighboring contact residues facilitated by this mutation. Additionally, the light chain (VL) of DIII030UCA paired with bnAb DH1030.1VH had no binding, in contrast to DIII030UCA59VL + DH1030.1VH, suggestive of VL gene mutations in bnAb maturation.
Conclusion: The DH1030 maturation pathway occurs via a series of VH and VL mutations, including HCDR2-G56R and HCDR3-S114P.

371 An Efficient Envelope Genotypic Assay to Identify bNAb Susceptibility in Infants with HIV
Kayla E. Delaney1, Mary F. Kearney1, Philipp A. Bester1, Nicola Greteze2, Susan Engelbrecht3, Carlo Gianguito4, Paolo Rossi5, Shaun L. Barnabas6, Moira J. Spyer3, Mathias Lichterfeld3, Alfredo Tagaro1, Carl Lombard5, Mark E. Cotton2, Gert U. van Zyl5, for the EPICAL Consortium

Stellenbosch University, Cape Town, South Africa, National Cancer Institute, Frederick, MD, USA, 1University of Free State, Bloemfontein, South Africa, University of Padua, Padova, Italy, 2Bambino Gesù Children’s Hospital, Rome, Italy, 3University College London, London, United Kingdom, 4Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, USA, 5Hospital Universitario de La Jolla, Madrid, Spain

Background: HIV-1 envelope (Env)-specific broadly neutralizing antibodies (bNAbs) have several potential clinical benefits including in infants: Infants have limited tolerable antiretroviral treatment (ART) options, given daily for prevention and twice daily for treatment. In contrast, bNAbs with modified Leucine-Serine (LS) Fc receptors permit infrequent subcutaneous dosing. Moreover, unlike ART, bNAbs may facilitate viral reservoir reduction and contribute to functional cure. However, data on HIV-1 Env evolution and bNAb susceptibility in perinatally infected infants are limited.

Methods: The evolution of Clade C Env in five infants (4 female) born with HIV-1 and with intermittent viraemia despite early ART was investigated over 19.3 (range: 16.9 - 21) months. A single-genome sequencing approach using Oxford Nanopore Technologies (ONT) was developed and validated using Sanger Sequencing as reference. In short, consensus sequences were constructed for each single genome using NECAT, a published bioinformatics pipeline that corrects ONT error. Defective individual genomes containing stop codons were excluded. The susceptibility of the remaining genomes to 33 bNAbs were investigated using the bNAb Resistance Predictor (bNAb-Rep) machine learning algorithm.

Results: The intra-patient average pairwise distances (APD) ranged from 0.08% - 1.29% (median: 0.43%). Different evolutionary patterns were observed. Env length variation emerged in 4 out of 5 infants. Phylogenetic trees showed temporal structure in four cases with new variants emerging either from majority or minority populations or apparent ancestral or archived variants. All variants were identified as CCR5-tropic by three genotypic prediction models. Predicted bNAb susceptibility showed much higher inter-patient than intra-patient variability with PGDM1400, PGT128 and 3BC117 (Table 1) predicted to have the highest susceptibilities overall.

Conclusions: As phenotypic bNAb susceptibility testing is costly and has low reproducibility, we developed an efficient Env genotypic assay, combining single-genome sequencing with ONT to accommodate Env sequence length variation. Applying our workflow, the predicted susceptibility to bNAbs varied across individuals due to high levels of inter-patient Env diversity. Early ART-treated infants often have viraemia due to adherence challenges. However, early intra-patient Env evolution was limited and unlikely to impact bNAb susceptibility. Updated prediction algorithms require validation across HIV-1 subtypes.

Table 1: Investigation of HIV-1 Env of 15 infants and the highest predicted bNAb susceptibilities

<table>
<thead>
<tr>
<th>Patient</th>
<th>Duration of observation (months)</th>
<th>Env APD (%)</th>
<th>Length variation (base pairs)</th>
<th>Most susceptible bNAbs</th>
<th>Probability susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA-TY012</td>
<td>16.9</td>
<td>1.02</td>
<td>2,550 - 2,571</td>
<td>PGDM1400</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PGT128</td>
<td>0.99</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10-1074</td>
<td>0.85</td>
</tr>
<tr>
<td>SA-TY015</td>
<td>10.5</td>
<td>0.00</td>
<td>2,500</td>
<td>PGDM1400</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H16</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VRC13</td>
<td>0.71</td>
</tr>
<tr>
<td>SA-TY025</td>
<td>10.1</td>
<td>0.38</td>
<td>2,565 - 2,598</td>
<td>PGT128</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10-1074</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3BC117</td>
<td>0.89</td>
</tr>
<tr>
<td>SA-TY032</td>
<td>19.3</td>
<td>1.29</td>
<td>2,568 - 2,599</td>
<td>3BC117</td>
<td>0.88</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>PO8</td>
<td>0.99</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>PGDM1400</td>
<td>0.93</td>
</tr>
<tr>
<td>SA-TY034</td>
<td>21.9</td>
<td>0.63</td>
<td>2,553 - 2,599</td>
<td>PGDM1400</td>
<td>0.96</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>PG1145</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PO8</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*APD - average pairwise distance
**Predicted by bNAb-Rep algorithm

372 Mycobacterium tuberculosis Infection Dampens the HIV-1 Antibody Response
Marius Zeeb1, Chloé Pasini1, Irene A. Abela1, Katharina Kusejko2, Sonja Hartnaeck3, Julia Notter3, Hansjakob Furrer3, Matthias Hoffmann3, Hans Hirsch3, Alexandra Calmy1, Enos Bernasconi1, Hudlyrich F. Gunthard1, Roger Koyou3, Alexandra Trkola4, Johannes Nemeth1, 1University Hospital Zurich, Zurich, Switzerland, 2University of Zurich, Zurich, Switzerland, 3St Gallen Cantonal Hospital, St Gallen, Switzerland, 4University Hospital Basel, Basel, Switzerland

Background: In prior investigations, we uncovered the impact of Mycobacterium tuberculosis (MTB) infection on the innate immune system in people with HIV (PWH). We showed an improved ability to control HIV and less susceptibility to opportunistic infections in untreated PWH. Intriguingly, it also heightened the risk of non-communicable diseases such as diabetes mellitus. Our exploration of the transcriptome in PWH, with and without MTB...
infection, unveiled gene alterations associated with innate immunity and B cell upregulation. Additionally, another group showed that active TB enhances immune responses. Here we extend these investigations and determine the effects of MTB infection on the anti-HIV-1 antibody (Ab) response. 

**Methods:** Using data from the Swiss HIV Cohort Study and the Swiss 4.5K neutralization Screen (Rusert, Nat Med, 2016), we assessed immune responses in PWH dependent on MTB status (excluding active TB and preventively treated). We examined two aspects: (i) plasma neutralization against eight HIV strains (activity/breadth score 0 to 24), and (ii) relative plasma Ab (IgG1/12/3) binding (score 0 to 1) to 20 HIV antigens. We determined the impact of MTB status by tobit and linear regression adjusting for ethnicity, age, sex, CD4, HIV viral load, viral diversity, and infection duration. We employed Bayesian networks to elucidate interactions between factors, particularly disentangle direct and indirect effects.

**Results:** Our analysis included 2,823 PWH (211 MTB infected). MTB infection was associated with a -0.74 (1.44, -0.05) neutralization reduction in multivariable tobit regression. Moreover, MTB infection was associated with a -0.04 (-0.07, -0.01) binding decrease of epitopes/IgG classes (IgG2 trimer, IgG1 gp140, IgG1 V3), previously shown to be predictive of high neutralization. Bayesian network analysis (Figure) showed MTB infection was associated with a -0.36 (-0.63, -0.08) log10 viral load decrease. In turn, one log10 viral load increase, was associated with a 0.11 (0.03,0.19) neutralization increase.

**Conclusion:** Consistent with our previous findings, our results underscore the immediate impact of MTB infection in reducing HIV-1 viral load. The resulting decreased exposure to HIV-1 antigen is likely the cause of the observed attenuated HIV-1 antibody response. While a modest decrease in binding Abs may be of limited consequence, a reduced ability to induce neutralizing Abs in untreated HIV and MTB co-infection may potentially counteract the gains in HIV-1 control induced by MTB.

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374 Temsavir Treatment Enhances bNAb Recognition and Subsequent Clearance of HIV-1–Infected Cells


**Background:** HIV-1 gp120 is a viral envelope glycoprotein (Env) expressed on the surface of infected cells which renders it an attractive target for the clearance of the infected cells. However, Env is metastable and presents in multiple conformations, making it challenging for antibody-mediated clearance. Temsavir (TMR), the active form of the HIV-1 attachment inhibitor fostemsavir, binds to and stabilizes Env in a 'closed' conformation that may occur at much higher concentrations than neutralization and that can infect humans (SARS-CoV-1 and SARS-CoV-2). Over the past 20 years, Sarbecoviruses represent a subgenus of coronaviruses including SARS-CoV-1 was responsible for an outbreak in Asia while SARS-CoV-2 initiated a pandemic in Europe. The SARS-CoV-2 strain is thought to be transmitted among people, which is also consistent with the large number of cases reported in Wuhan, China. The SARS-CoV-2 strain is spread primarily through respiratory droplets and has an incubation period of 2-14 days. The virus can also be spread through contact with infected individuals or surfaces. Early studies on SARS-CoV and SARS-CoV-2 have shown that the virus can infect and replicate in cells of the respiratory system, gastrointestinal tract, and other tissues. In the respiratory system, the virus can infect alveolar cells and lead to pneumonia. In the gastrointestinal tract, the virus can infect enterocytes and lead to diarrhea. These findings have implications for the treatment and prevention of SARS-CoV-2 infection.

**Methods:** To understand whether TMR can modulate bNAb binding to Env on infected cells, we conducted experiments with primary CD4+ T cells isolated from infected cells through antibody-dependent cellular cytotoxicity (ADCC). This synergistic effect of N6 and TMR is most prominently observed on infected cells that maintain CD4 expression, the cells that are otherwise difficult to target by N6 and other bNabs alone. The enhanced bNAb binding is diminished when tested against strains with reduced TMR sensitivity (clade AE), suggesting that it is an on-target MOA. We observed decreased bNAb binding to CD4 downregulated infected cells with some viruses. However, this effect only occurs at much higher TMR concentrations than required for neutralization and is not consistent across the panel. Alternatively, we saw significantly increased bNAb binding to the CD4 downregulated cells in 1/3 of isolates that are tested. Further analysis of HIV Env from infected cells demonstrated that the altered bNAb binding is not attributed to the modified gp160/gp120 processing by TMR.

**Conclusion:** We have demonstrated for the majority of viruses tested that TMR treatment leads to enhanced bNAb binding to the infected cells that maintain CD4 expression, and the reduced bNAb binding to the CD4 downregulated infected cells occurs at much higher concentrations than neutralization. These results suggest that combination of bNAb and TMR can expand the population of HIV-1 infected cells susceptible to bNAb-mediated clearance and may increase the likelihood of reservoir reduction in the clinical setting.

375 AAV-1, -8, and -9 Seroprevalence in Healthy Donors and People Living With HIV in Sub-Saharan Africa

Giselle Lopez Fernandez1, Daniel O’Hagan1, Siddhartha Shandilya2, Dorinda Mukura3, Tinashe Chideme1, Alfred Katema4, Rodney Goreraza5, Mookho Malahleha6, Zoe Moodie7, Mauricio A. Martins8

1The Herbert Wertheim UF Scirps Institute for Biomedical Innovation & Technology, Jupiter, FL, USA, 2University of Zimbabwe, Harare, Zimbabwe, 3Synergy Biomed Research Institute, London, United Kingdom, 4Fred Hutchinson Cancer Center, Seattle, WA, USA, 5The Herbert Wertheim UF Scirps Institute for Biomedical Innovation & Technology, Gainesville, FL, USA

**Background:** Adeno-associated virus (AAV)-vector delivered monoclonal (m) HIV-specific broadly (b) neutralizing (n) antibodies (Abs) holds promise for treating HIV infection. Because AAV is non-pathogenic and its genome persists in host cells, successful AAV/bnAb transduction of long-lived cells, such as myocytes, can result in continuous bnAb expression for years, possibly decades. We set out to address a critical barrier to the clinical use of AAV/bnAb vectors: the high prevalence of pre-existing anti-AAV nAbs in humans. One way to maximize the impact of AAV/bnAb therapies is to determine the seroprevalence of muscle-tropic AAV capsids in areas with high incidence of HIV, so that people living with HIV (PLWH) who are seronegative for these AAV capsids can potentially benefit from AAV/bnAb therapies without any additional intervention. AAV epidemiology is well documented in developed countries but nearly absent for sub-Saharan Africa, home of most PLWH.

**Methods:** To address this deficit, we partnered with the HVTN (HIV Vaccine Trials Network) and ACTG (AIDS Clinical Trials Group) to establish the prevalence and titers of anti-AAV nAbs in South Africa and Zimbabwe. We used HEK293T cells and luciferase-expressing vectors to screen sera from 300 healthy adult donors (HD) and 277 PLWH for nAbs against AAV-1, -8, and -9.

**Results:** First, we screened sera at a 1:20 dilution and found that 15%, 79%, and 77% of HD samples displayed <50% neutralization of AAV-1, -8, and -9, respectively. We found no significant sex-specific differences in the prevalence of anti-AAV nAbs in this cohort. Among PLWH, 17%, 72%, and 73% of samples exhibited <50% neutralization of AAV-1, -8, and -9, respectively. Next, we determined the midpoint titers of anti-AAV-9 nAbs in the samples that exhibited >50% neutralization at a 1:20 dilution (i.e., AAV-9+). We selected AAV-9 for this in-depth analysis because of its superior performance to AAV-1 and -8 in promoting persistent mAb expression in nonhuman primates following intramuscular (IM) administration. Among HD, 75% of AAV-9+ donors exhibited titers of anti-AAV-9 nAbs below 1:160, a level that is not thought to impair IM AAV transduction in primates. We are currently determining the titers of anti-AAV9 nAbs among the AAV-9+ PLWH.

**Conclusion:** In summary, nearly three quarters of HD and PLWH in South Africa and Zimbabwe could be eligible to participate in clinical trials of bnAb-encoding AAV vectors delivered by the IM route.

376 Machine Learning-Guided Generation of a Combination of Broadly Neutralizing Sarbecovirus Antibodies

Grace Marden, Kimberly Schmitt, Hongru Li, Alex Ramos, Erin Carlin, Nadine Shaban, Geetika Sharma, Monica Menzenksi, Anne Jecrois, Gevorg Grigoryan, Adam Root, Heather Van Epps, Kristen Hopson, Darja Hazuda, Francesco Borriello, 5Marden

Institute for Biomedical Innovation & Technology, Gainesville, FL, USA

**Background:** Sarbecoviruses represent a subgenus of coronaviruses including strains mostly circulating in bats and at risk of zoonotic spillover as well as strains that can infect humans (SARS-CoV-1 and SARS-CoV-2). Over the past 20 years, SARS-CoV-1 was responsible for an outbreak in Asia while SARS-CoV-2 initiated a pandemic in Europe. The SARS-CoV-2 strain is thought to be transmitted among people, which is also consistent with the large number of cases reported in Wuhan, China. The SARS-CoV-2 strain is spread primarily through respiratory droplets and has an incubation period of 2-14 days. The virus can also be spread through contact with infected individuals or surfaces. Early studies on SARS-CoV and SARS-CoV-2 have shown that the virus can infect and replicate in cells of the respiratory system, gastrointestinal tract, and other tissues. In the respiratory system, the virus can infect alveolar cells and lead to pneumonia. In the gastrointestinal tract, the virus can infect enterocytes and lead to diarrhea. These findings have implications for the treatment and prevention of SARS-CoV-2 infection.

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**Conclusion:** In summary, nearly three quarters of HD and PLWH in South Africa and Zimbabwe could be eligible to participate in clinical trials of bnAb-encoding AAV vectors delivered by the IM route.
global pandemic and remains a continuous health threat especially for vulnerable populations. There is still the need to develop therapeutic measures that can effectively control human sarbecoviruses and potential zoonotic spillover events, thereby preventing future epidemics/pandemics. Several monoclonal antibodies have been discovered with broad neutralizing activity against sarbecoviruses by targeting conserved regions of the spike proteins such as class 3 and 4 RBD regions and S2 fusion machinery. Viral escape mutations accrued in the SARS-CoV-2 spike protein have rendered most of the antibodies targeting RBD regions ineffective. In addition, antibodies targeting the S2 fusion machinery have relatively low neutralization potency compared to anti-RBD antibodies and therefore their therapeutic utility has not been realized.

Methods: Here we used a machine learning-guided protein engineering approach to optimize broadly neutralizing antibodies against sarbecoviruses. We screened computationally designed sequence sets for binding to spike proteins, neutralization of pseudoviruses and developability parameters to select lead molecules. Additional characterization of lead molecules as single agents and combination included: epitope mapping through cryo-EM and X-ray crystallography, assessment of binding affinities to spike proteins, neutralization of pseudoviruses and live viruses, in vitro escape experiments and in vivo hamster challenge with SARS-CoV-2 BA.2.

Results: We rescued the neutralizing activity of a previously described class 4 anti-RBD antibody against Omicron variants. We also improved neutralization potency and efficacy of a previously described antibody with pan-sarbecovirus activity targeting the S2 stem helix peptide. Finally, we demonstrate that the combination of these optimized antibodies improves both neutralization of SARS-CoV-2 variants in vitro and suppression of viral replication in a hamster challenge model.

Conclusion: Our work highlights the successful use of machine learning-guided protein engineering for optimization of anti-viral antibodies and supports further evaluation of the described antibody combination for epidemic/ pandemic response and preparedness.

Safety and Immunogenicity of Month 30 Boost of ALVAC-gp120/MF59 Preventive HIV Vaccines

Vimia Naicker1, Fatima Laher2, Kelly Seaton3, Stephen C. De Rosa4, Lynn Morris5, Nonhlanhla N. Mkhize6, Linda-Gail Bekker7, Mokho Malahleh8,9, Kathy T. Mngadi9, Jack R. Hepstall10, David C. Montefiori11, Juliana Mc Erath12, Georgia D. Tomaras13, Zoe Moodie7,14 for the HVTN 100 Study Team

1South African Medical Research Council, Durban, South Africa, 2Perinatal HIV Research Unit, Soweto, South Africa, 3Duke University, Durham, NC, USA, 4Fred Hutchinson Cancer Center, Seattle, WA, USA, 5University of the Witwatersrand, Johannesburg, South Africa, 6National Institute for Communicable Diseases, Johannesburg, South Africa, 7Diamond T ATP Research Foundation, Cape Town, South Africa, 8Syngene Biomed Research Institute, East London, UK, 9The Aurum Institute, Johannesburg, South Africa, 10Duke University School of Medicine, Durham, NC, USA, 11Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Background: HVTN 100, a phase 1-2 preventive HIV vaccine trial in South Africa, administered subtype C-containing ALVAC HIV (pCP2938) at months 0 and 1, and ALVAC-HIV with bivalent subtype C gp120/MF59 at months 3, 6, and 12 in Part A. IgG binding antibody and T-cell responses were similar or greater at month 12.5 compared to month 6.5 then waned by month 18. In Part B we boosted after an 18-month interval at month 30.

Methods: Part B vaccinations were administered to eligible Part A participants, randomized to either ALVAC-HIV + gp120/MF59(n=32), or gp120/MF59 alone(n=31) and placebo group was re-administered placebo(n=7). At months 30, 35 and 36, we measured envelope (Env)-specific serum binding antibodies by binding antibody multiplex assay (BAMA) and HIV-specific CD4 T-cell responses by intracellular cytokine staining assay, and neutralization by reductions in Tat-regulated luciferase reporter gene expression in TZM-bl cells.

Results: Vaccine groups had an acceptable safety profile based on reactogenicity, adverse events and laboratory tests. There were no statistically significant differences in IgG binding antibody response rates or magnitudes between the two vaccine groups for any gp120, gp140, or V1V2 antigens at any timepoint. Vaccine groups had positive IgG responses to all V1V2(n=27), gp120(n=11) and gp140 antigens(n=9) at all timepoints but were more common for gp120 and gp140 antigens and highest at month 30.5. Booster vaccination restored the magnitude-breadth IgG response to V1V2 antigens at Month 30.5 which waned by month 36 with median area under the curves (AUCs) for combined vaccine groups 0.13, 2.55 and 0.40 for months 30, 30.5 and 36 respectively (Fig 1). Month 30 boosting increased magnitude and durability of tier 1A neutralization responses but did not induce tier 2 neutralization responses. Month 30.5 tier 1A response magnitudes for the pooled vaccine group increased significantly compared to month 12.5, median 331 versus 756, p<0.0001 for TV1/c8.2; 886 vs. 1763, p<0.0001 for MW965.2b). CD4+ Env responses, rate and magnitude, to vaccine-matched antigens were seen at month 30, boosted at month 30.5, waned by month 36, with no significant differences between vaccine groups.

Conclusion: Booster vaccination with gp120/MF59 given alone or with ALVAC after an 18-month interval was safe and induced binding, tier 1A neutralization and CD4+ T-cell responses similarly in both vaccine groups. Late boosting may increase breadth of responses and restore V1V2 binding antibody responses.

Adenosine Deaminase-1 Enhances HIV-Specific Responses to a HIV Trimeric Envelope DNA Vaccine

Gina Usimana1, David Joyner2, Emily Konopka3, Roshell Mair3, Gabriela Canziani4, Philip Barnette5, Ivan del Moral-Sánchez5, Tom Bijl6, Jonne Snitselaar7, Kyra Wolosczuk8,9, Irwin M. Chaiken10, Ann J. Hessell10, Rogier W. Sanders11, Elias K. Haddad12, Michele A. Kutcher13, 1Drexel College of Medicine, Philadelphia, PA, USA, 2Oregon Health and Sciences University, Portland, OR, USA, 3University of Amsterdam, Amsterdam, Netherlands

Background: Human immunodeficiency virus (HIV) remains a prominent global health threat for which no prophylactic vaccine is available. Extensive research efforts have resulted in immunogens, including S0SP trimers, that closely mimic the native envelope (Env) glycoprotein conformation and consistently induce autologous neutralizing responses. Recently, a novel triple tandem trimer (TTT) platform has been used to generate a plasmid encoding Env immunogen (pBG505-TTT) that expresses only as trimers, making it more suitable for nucleic acid vaccines. We have demonstrated that adenosine deaminase-1 (ADA-1) is critical to the T follicular helper (TFH) function and survival. In fact, ADA-1-induced TFH function that improved the magnitude and durability of both cellular and humoral vaccine immune responses in vivo. We therefore hypothesized that ADA-1 combined with the improved HIV envelope antigen pBG505-TTT would result in enhanced qualitative and quantitative humoral and cellular HIV specific responses.

Methods: Mice were immunized intramuscularly with 1, 5, or 10μg of pBG505-TTAlone or were co-immunized with 10μg of plasmid encoded adenosine deaminase-1 (pADA). A separate group of mice were immunized with both DNA as described and 10μg of trimeric Env recombinant protein (rBG505-S05P) with adjuvants Alum or MF59. Mice were bled on day 21 post 2 immunizations (D21P2) and day 14 post 3 immunizations (D14P3) to evaluate humoral responses via ELISA, surface plasmmon resonance, and neutralization assays. Cellular responses were evaluated on D14P3 via ELISPOT assays, T cell intracellular cytokine staining and antigen specific memory B cell staining via flow cytometry.

Results: Mice co-immunized with pADA and 1μg pBG505-TTT displayed significantly increased Env specific antibody titers as early as D21P2. pADA and
10μg pBG505-TTT-co-immunized mice exhibited Env specific antibody with enhanced affinity and increased Env specific memory B cells when compared to non-adjuvant counterparts. Mice immunized with 10μg pBG505-TTT, pADA, rBG505-SOSIP and alum exhibited enhanced neutralization compared to their non-pADA adjuvant counterparts. Mice receiving pADA and 1μg or 5 μg pBG505-TTT exhibited enhanced Env specific T cell activation, cytokine polyfunctionality, and degranulation.

**Conclusion:** These data demonstrate that pADA enhances both cellular and humoral immunity in a dose sparing manner against HIV Env making it a promising adjuvant for HIV targeting vaccines.

### 379 Sex-Based Differences in Antibody Responses Induced by a Native-Like HIV-1 Envelope Trimer Vaccine

Emma Reis, Karlijn van der Straaten, Marius Liesdek1, Annelou L. van der Veen, Marloes Grobben, Hongmei Gao3, Kelli Greene3, David C. Montefiori3, Maarten Soeters3, Michelle J. Kluowens1, Jan M. Prins1, Marit van Gils3, Rogier W. Sanders1, Godelieve J. de Bree

1University of Amsterdam, Amsterdam, Netherlands, 2Amsterdam UMC, Location University of Amsterdam, Amsterdam, the Netherlands, 3Duke University School of Medicine, Durham, NC, USA

**Background:** A protective vaccine would be the most powerful tool towards reducing HIV-1 infections worldwide and sustainably ending the AIDS epidemic as a public health threat. We assessed the safety and immunogenicity of ConM SOSIP.v7, a native-like envelope trimer vaccine based on an HIV-1 group M consensus sequence, in HIV-negative adults.

**Methods:** 24 individuals were enrolled in a phase 1 clinical trial to receive three doses of adjuvanted ConM SOSIP.v7 (baseline, eight and 24 weeks) and followed-up for one year thereafter. Out of 23 per-procol vaccine recipients, 10 received a one-fifth fractional third dose, aimed to increase B cell somatic hypermutation. Outcomes included ConM-specific total immunoglobulin G (IgG), IgG subclasses, pseudovirus neutralisation and single B cell sequencing. Levels of sex hormones were measured over time.

**Results:** The majority of adverse events were mild to moderate, self-limiting and similar between dosage groups and sexes. No serious adverse events were reported. Similar to non-human primates, adjuvanted ConM SOSIP.v7 was consistently capable of inducing an autologous neutralising antibody response in humans, which remained detectable in half of the participants six months after the final vaccination. Antibody binding to several heterologous envelope proteins could be detected in the majority of participants, but no neutralisation breadth. No differences in serological outcomes could be attributed to the fractional third dose. Female born participants had an earlier increase in neutralising antibodies than males and had a 6.3-fold higher neutralisation titer post third vaccination. Sex-based differences in IgG1 and IgG4 subtype responses were also observed. Subtle correlations, both positive and negative, were seen between ConM-neutralisation, IgG3 and IgG4 levels on one hand, and oestradiol and testosterone on the other hand, but the small sample size limits drawing strong conclusions. Single B cell sequencing should reveal whether the fractional dose and/or sex influence B cell affinity maturation.

**Conclusion:** The adjuvanted ConM SOSIP.v7 native-like trimer vaccine is safe and elicits a robust strain-specific neutralising response in nearly all recipients, making it an interesting boosting immunogen candidate. Females responded earlier and had a striking 6.3-fold higher neutralisation titer after the final vaccination. This study highlights that sex-based differences should be taken into consideration when assessing HIV-1 vaccine candidates and adjuvants.

### 380 IgG and Fc Receptor Genetic Variation Associates With Functional Antibody Responses in HVTN 108

Song Young Oh1, Jaehyeon Lee2, C. Alex Gollings1, Matthew D. Gray, Leonidas Stamatatos2, Madison M. Means, Matthew D. Gray

1Duke University, Durham, NC, USA, 2Duke University School of Medicine, Durham, NC, USA

**Background:** The HVTN108 trial evaluated the immunogenicity of a DNA prime, adjuvanted protein boost vaccine regimen in the US and South Africa. We defined the IgG antibody Fc and Fc receptor (FcR) genotypes in the study population to test our hypothesis that IgG and FcR genetic variation can affect vaccine-elicted functional antibody responses.

**Methods:** HVTN108 enrolled 334 participants in treatment (n=310) and placebo (n=24) groups. The genotypes of IgG Fc and FcR were determined by targeted PCR amplification and Illumina next-generation sequencing. Vaccine-elicited functional antibody responses, including binding antibody multiplex assay (BAMA), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP) assays, were measured by HVTN core labs. Pearson correlation analysis of IgG and FcR genetic variants was used to identify potential linkage disequilibrium (LD). Relationships between genotypes and antibody responses were identified by linear regression controlling for treatment group and region. Variants were deemed to be associated with antibody responses when the p-value was below 0.05 and the Benjamin-Hochberg false discovery rate (FDR) adjusted value below 0.02.

**Results:** In the study population, there was evidence of LD within IgG and FcR alleles, but no strong correlations between specific IgG and FcR alleles (all r values were <0.5). The distribution of many polymorphisms significantly differed between the US and South Africa. Within the subset of the cohort tested for functional antibody responses (IgG, n=41; FcR, n=55), we determined that IgG genotypes such as IgG1_12 (p=0.010, p=0.027), IgG3_11 (p=0.028, p=0.050), IgG2_02 (p=0.032, p=0.102), IgG4_07 (p=0.064, p=0.133), and others were associated with vaccine-elicited ADCC antibody titers and activity peaks when corrected for vaccine group and regional effects. In the same way, we identified that the FCER1A rs2427827 had a significant association with higher peak ADCC activity and the FCGR2A rs1801274 mutation was associated with a higher antibody binding to a subtype C V1V2 antigen.

**Conclusion:** Multiple IgG genotypes and FcR mutations were associated with ADCC and binding antibody levels after HVTN108 vaccination when controlling treatment and region. Genetic variation in both antibodies and FcRs contributes to vaccine-induced humoral responses but there were significant regional differences in their distribution, suggesting regional development of HIV vaccines may be scientifically appropriate.

### 381 Developing Novel HIV-1 Env Proteins That Efficiently Engage the VRC01-class Precursors in Clinical Trials

Parul Agrawal, Anuradha Agrawal, Kallur, Madison M. Means, Matthew D. Gray, Leonidas Stamatatos1, Guido Ferrari2, One Dintwe

1University of KwaZulu-Natal, Durban, South Africa, 2Duke University, Durham, NC, USA

**Background:** HVTN108 evaluated the immunogenicity of a DNA prime, adjuvanted protein boost vaccine regimen in the US and South Africa. We defined the IgG antibody Fc and Fc receptor (FcR) genotypes in the study population to test our hypothesis that IgG and FcR genetic variation can affect vaccine-elicted functional antibody responses. We previously reported that specific modification of the V1V2 domain resulted in increased neutralising antibody responses. These antibodies target the CD4 binding site (CD4-BS) of the HIV-1 envelope glycocapsid (Env) and have been shown to prevent HIV-1 acquisition in two phase 3 clinical trials. Based on new structural information of Envs, we have now identified additional N-linked glycosylation site mutations that allow Envs from different clades to bind V1V2 domain efficiently. This is potentially why Env immunization has so far failed to elicit V1V2 domain-specific antibodies. We previously reported that specific modification of the V1V2 domain resulted in increased neutralising antibody responses. These antibodies target the CD4 binding site (CD4-BS) of the HIV-1 envelope glycocapsid (Env) and have been shown to prevent HIV-1 acquisition in two phase 3 clinical trials. We previously reported that specific modification of the V1V2 domain resulted in increased neutralising antibody responses. These antibodies target the CD4 binding site (CD4-BS) of the HIV-1 envelope glycocapsid (Env) and have been shown to prevent HIV-1 acquisition in two phase 3 clinical trials.
382 Enhancing HIV Vaccine Efficacy via Langerhans Cell-Targeted Env Trimers
Adele Hammoudi1, Mathieu Sureauaut1, Florencio Picard1, Jade Legros1, Emma Sichreret1, Borys Pedenkoz2, Christiane Moop3, Winfried Weissenhorn4, Mireille Centlivre1, Véronique Godot1, Yves Levy1, Sylvain Cardinaud1

1Institut Mondor de Recherche Biomédicale (IMRB), Créteil, France; 2Institut de Biologie Structurale, Grenoble, France; 3University of Strasbourg, Strasbourg, France; 4Grenoble Alpes University, Grenoble, France

Background: Developing an effective HIV-1 vaccine is contingent on generating protective antibodies (Abs). Novel antigen delivery methods are needed to enhance immune responses. One promising approach involves directing antigens to dendritic cells (DC) through fused monoclonal antibodies (mAbs) to amplify both cellular and humoral responses. Previous studies have shown success in targeting skin Langerhans cells (LC) with anti-Langerin mAbs fused to HIV-1 Envelope (LC.Env), inducing antigen-specific humoral responses in mice and human LC/TB co-cultures. In this study, we aimed to refine LC targeting by designing three Env monochains instead of two (LC3.Envs) mimicking natural Env conformation. We aim to investigate whether these new trimeric Env constructs could stimulate germinal center (GC) and Tfh cell reactions, ultimately leading to the production of Env-binding IgG and HIV neutralizing antibodies (NeutAb) in vivo.

Methods: B6 mice were intradermally immunized with LC3.Env or control LC.Env mAb (5 mcg of Env antigen) at day (D) 0 and D21, without adjuvant. Antibody (magnitude and affinity) and cellular responses were assessed post-prime (PP) and post-boost (PB) using Luminox and FACS, respectively. GC/Tfh reactions in draining lymph nodes (dLN) were monitored through immunofluorescence at various times. Additionally, rabbits were subcutaneously immunized with 50 mcg LC3.Env without adjuvants, and serum NeutAb levels were evaluated.

Results: Compared to LC.Env, LC3.Env: (i) elicited a rapid and potent Env-IgG response, with mean titers measuring 22- and 37-fold higher at D14 PP and D7 PB, respectively. (ii) enhanced the avidity of anti-Env IgG, resulting in an index increase of 12% and 36% at D14 PP and D7 PB, respectively. This was accompanied by a marked expansion of Tfh and GC B cells (GL-7+/Fas+) at D7 PB. (iii) swiftly induced the formation of structured germinal centers in dLN, indicative of a robust immune response. Significant Tier-1 NeutAb induction was observed in rabbits immunized with LC3.Env.

Conclusion: This study underscores that HIV Env antigen can be adeptly targeted to LC as a transmitter, intensifying both the magnitude and quality of humoral responses without using any adjuvant and after only two shots. In addition, the strategy of targeting SOSIP-like trimeric Env to LC holds substantial promise for eliciting broadly neutralizing antibodies (bNAb) against HIV and will require an optimization of the vaccination regimen.

383 Mucosal HIV Vaccine Targeting Host Epithelial Stem Cells for Long-Term Immunity
Robert White1, Vida Hodara1, Patrice Frost2, Pamela A. Kozlowski3, Francois J. Villinger4, Marie-Claude Gauduin1

1Texas Biomedical Research Institute, San Antonio, TX, USA; 2Southwest National Primate Research Center, San Antonio, TX, USA; 3Louisiana State University, New Orleans, LA, USA; 4University of Louisiana at Lafayette, Lafayette, LA, USA

Background: HIV transmission occurs predominantly across mucosal surfaces; an ideal vaccine should be to target HIV at mucosal entry sites to prevent infection. We developed a SIV single-cycle replication-deficient vaccine under the control of the invovlucrin promoter, which was tested for its ability to drive SIV expression in terminally differentiated epithelial cells, induce mucosal immune responses, offering protection against SIV.

Methods: Sixteen macaques (8 females, 8 males) were immunized (1 dose: 50ng p27=3x10^11 RNA copies, atrumatic, needleless) at week 0 and monitored over time for specific immune responses in blood, mucosal secretions, and various lymphoid/non-lymphoid tissues. At weeks 12 (group 1) or 24 (group 2), animals were challenged with repeated low-doses SIVmac239 (intravaginal, 2500 TCID_50/ml; intrarectal at 300 TCID_50/ml) 4 females and 4 males per group. Sixteen additional animals (8 females, 8 males) served as unvaccinated SIV-infected Controls.

Results: Within two weeks post-vaccine, strong mucosal antibody responses (IgG, IgA) and specific CD8+ T-cells were detected. Immunohistofluorescence revealed antigens-expression in epithelia upper-layers. SIVenv was detected via anti-gp120 immunePET/CT scans in female vagina and draining-LN as well as in male rectal and transversal. SIV-challenges demonstrated a significant delay and/or lower viremia (2-3 logs-reduction, peak; 4-5 logs-reduction, set-point) to undetectable viremia 10-20 weeks post-SIV in vaccinees. Robust SIV-specific T-cell responses were also detected in blood, lymph-nodes, and mucosa tissues. Controls had high viremia (log_{10} 7.2-8.7 viral-RNA copies/ml, peak) and significant gut CD4+ T-cells depletion. We demonstrated a positive correlation between mucosal and systemic T-cell responses and control of viremia, and inverse associations between viremia and post-challenge vaginal antibody responses. All vaccinees manifested durable aviremic SIV-control for 2 years when CD8-depletion was performed. The dramatic fall in viremia coincided with the CD8+ T-cells recovery and significant increase of SIV-specific responses.

Conclusion: The study demonstrated the efficacy of an epithelial stem cell-based vaccine to serve as antigen delivery to generate specific mucosal antibody and cellular immune responses leading to significant delays in infection followed by rapid and durable plasma viral control to undetectable.

384 Naive B-Cells From Unvaccinated Rhesus Macaques Cross-React With HIV gp140 Protein
Michelle Premazzi Papa1, Andrew Wilson2, Rosemarie Mason1, Jennifer A. Manuzak1, Nichole R. Klatt2, Rebecca M. Lynch2

1George Washington University, Washington, DC, USA; 2National Institutes of Health, Bethesda, MD, USA; 3Tulane National Primate Research Center, Covington, LA, USA; 4University of Minnesota, Minneapolis, MN, USA

Background: HIV causes a chronic infection that is not cleared by the normal immune response, in part due to an ineffective early antibody response. Other groups have shown that early in infection, people with HIV-1 (PWH) develop anti-gp41 antibodies that cross-react with commensal bacteria but do not neutralize HIV, and that this same phenomenon occurs after vaccination. Here, we investigated if the baseline antibody response prior to HIV vaccination is comprised of antibodies targeting commensal bacterial that weakly target HIV, and if these antibodies are preferentially activated after subsequent infection.

Methods: To test this hypothesis, PBMC samples from a SIV HIV DNA vaccine (SIV gag, p55), HIV env (gp160), and gp140 trimeric protein and simian-human immunodeficiency virus (SHIV) challenge study in Rhesus Macaques (RMs; n=8) were collected prior to vaccination (week -7) and stained using an 11-color B cell panel. HIV-1 gp140-specific B cells from these SHIV-naive RMs were sorted and nested-PCR was performed using specific primers for IgG, IgM, IgA heavy chains, and Kappa and Lambda light chains. PCR products were sequenced and compared to germline using IMGT/V-QUEST. Selected sequences were cloned as IgG1 and tested for function.

Results: A total of 120 cells were sorted (ranging from 1-44 cells per RhM). 95 heavy chains were amplified, and 70 had productive ORF sequences. 56 out of 70 were IgM, heavy were IgA, and seven were IgG sequences. Analyzing light chains, a total of 110 were amplified and 63 had productive ORF sequences. 42 out 63 were Kappa and 21 were Lambda sequences. From these, 35 had productive heavy and light chain sequences. Fourteen cells that exhibited intermediate and high gp140 binding during cell sorting were selected for cloning. Monoclonal antibodies (mAbs) were generated and tested for binding to gp41, gp120, and gp140 proteins. Ten mAbs recognized gp41, two mAbs bound to gp120, and five recognized gp140. A total of four mAbs bound to MARCK14 with high and low affinities, the two with high affinity also bound to LPS (Fig 1). This data shows that these mAbs not only cross-react with bacteria but also bind to different cellular components suggesting that these are polyreactive antibodies.
Background: People living with HIV (PLWH) are recommended to be fully vaccinated against SARS-CoV-2. However, men with HIV-1 (MWH) respond to the COVID-19 vaccine with lower immunoglobulin binding and neutralization titers against SARS-CoV-2 than men without HIV-1 (MWOH). Although neutralization is an important protective mechanism against SARS-CoV-2, other mechanisms can play a key role in protection. Therefore, we assessed antigen-specific complement activation in vaccinated MWH and MWOH.

Methods: We quantified activation of the classical complement cascade through C3 deposition by antibodies specific for SARS-CoV-2 S1 and for the receptor binding domain (RBD) in MWH and MWOH from the MACS/WIHS Combined Cohort Study using a bead-based flow cytometry assay and capture C3/C3a ELISA.

Results: When matched on anti-S1 IgG titer, MWH had 10-fold greater C3 deposition than MWOH against the Washington (WA) and Omicron strains. However, the level of complement activation was proportional to the IgG titer. MWH thus, had lower C3 deposition against the RBD of both WA and Omicron strains without titer matching, which is consistent with MWH having lower vaccine induced antibody titters than MWOH. For both MWH and MWOH, C3 deposition against the RBD and full S1 proteins were comparable. However, C3 deposition against the Omicron RBD was lower than for the WA RBD, indicating lower affinity of antibodies binding to the variant RBD. These antibodies still induced a strong activation of the classical complement cascade, even as neutralization titers against the Omicron strain are low, indicating that non-neutralizing antibodies that bind to Omicron can mediate protection through complement activation. Finally, we found that the C3 levels significantly declined post-vaccination in MWH, concomitant with a significant increase in C3a levels in serum, suggesting an elevation of the baseline complement activity in this group.

Conclusion: Collectively, these data suggest that complement activation is a mechanism of protection against SARS-CoV-2 vaccination, and that it can have a greater impact on protection against SARS-CoV-2 variants in the absence of neutralizing antibody titers. It also suggests that antibodies with greater complement activation potency in MWH can compensate for lower antibody titers. These results highlight a critical mechanism of protection in MWH that can inform further booster vaccination schedules.
Immune Responses to an Original/BA.4-5 Bivalent Booster of SARS-CoV-2 mRNA Vaccine in PWH on ART
Matteo Augello, Valeria Boni, Roberta Rovito, Alessandro Tavelli, Camilla Tincaiti, Alessandra Vergori, Anna Maria Azzini, Elia Riggi, Andrea Antonini, Evelina Tacconelli, Antonella D’Arminio Monforte, Giulia Marchetti, for the VaultCONA-ORCHESTRA Study Group
1 University of Milan, Milan, Italy, 2Icona Foundation, Milan, Italy, 3Lazzaro Spallanzani National Institute for Infectious Diseases, Rome, Italy, 4University of Verona, Verona, Italy

Background: In the current Omicron era of COVID-19 pandemic, boosters with variant-adapted mRNA vaccines are recommended for fragile populations to provide broad protection against newly emergent SARS-CoV-2 variants. While previous studies showed enhanced neutralization against Omicron sub-lineages after a bivalent booster, data on T-cell immunity are limited, especially in PWH. We assessed humoral and T-cell responses to an original/BA.4-5 booster in this population.

Methods: PWH on ART receiving a bivalent booster as fourth dose were enrolled. We measured immune responses against WT and BA.4-5 virus: RBD-binding Abs (ELISA); RBD-blocking Abs (RBD-ACE2 binding inhibition assay); Th1/Tc1 cytokine (IFN-γ/TNF-α/IL-2)-producing and cytotoxic (CD107a+) CD4/CD8 T-cells (flow cytometry after PBMCs stimulation with spike peptides) before (T0) and 1 month after (T1) the 4th dose. Wilcoxon test was used for statistical analyses.

Results: We included 30 PWH who received the 4th dose at a median time of 14.5 (IQR: 13.5–15) months after the 3rd one. Median CD4 T-cell count was 790/μL (IQR: 598–929) and HIV-RNA <20 copies/mL. Demographic and viro-immunologic features are reported in Fig.1A. The 4th dose led to an increase of RBD-binding/blocking Abs against both WT and BA.4-5. Interestingly, when compared to WT, BA.4-5–specific humoral immunity was lower at both T0 and T1. Frequencies of Th1/Tc1 and cytotoxic CD4/CD8 T-cells against WT and BA.4-5 did not increase after the booster, with similar values of WT– and BA.4-5–specific responses both before and after the booster administration (Fig.1D–G). Despite stable frequencies, polyfunctionality of WT/BA.4-5–specific cells was increased after the booster, and yet the proportion of BA.4-5–specific polyfunctional IFN-γ+TNF-α+IL-2+/IFN-γ+TNF-α+IL-2– CD4 T-cells and IFN-γ+TNF-α+IL-2– CD8 T-cells were lower than WT–specific ones at all time-points.

Conclusion: In PWH on effective ART, an original/BA.4-5 bivalent booster increases RBD-binding/blocking Abs and T-cell polyfunctionality against both WT and BA.4-5. However, humoral and polyfunctional T-cell responses hold higher against WT than BA.4-5 both before and after the booster, suggesting immune imprinting to the wild-type strain. By showing a strengthened antigen-specific immune response, our data reinforce the importance of a variant-adapted mRNA boosters in PWH, possibly directed against the dominant mutated regions of the spike protein.

Immune Response to SARS-CoV-2 Omicron Breakthrough is a Recall of Vaccine-Induced Memory
Jernej Pusnik, Jasmín Zorn, Werner O. Monzon Pocasal, Kathrin Peter, Emmanuil Ospytschuk, Sabine Blaschke, Hendrik Streick
1 Bonn University Hospital, Bonn, Germany, 2University Medical Center Göttingen, Göttingen, Germany

Background: The COVID-19 vaccination campaign is considered the largest immunization program since World War II. However, effective vaccine designs. Here, we conducted a high-resolution mapping of the breadth and magnitude of T-cell responses to the entire SARS-CoV-2 proteome over a 2-year follow-up period in individuals with hybrid immunity.

Methods: We selected cryopreserved PBMCs from 38 healthcare workers, including 19 SARS-CoV-2 infected (CoV2+, tested at 124 days from symptoms onset, D50) and 19 uninfected (CoV2–) participants in the ProHEPIC-19 cohort study (NCT04885478). Longitudinal PBMC were available from 18 individuals after a 3- dose mRNA vaccination, including 13 CoV2+ (CoV2+Vac+, 825 DfSO) and 5 CoV2– who became infected during the follow-up period (Vac+CoV2+, 302 DfSO). We measured the breadth and magnitude of IFN-γ T-cell responses by ELISPot assay using a 15-mer overlapping peptide (OLP) library of 2,790 SARS-CoV-2 peptides in 100 pools using a Mega Matrix approach, and we deconvoluted the matrix using single peptides by ELISPot assay.

Results: We identified immunodominant T-cell responses to 13 regions across the SARS-CoV-2 proteome within S, Nsp3, NC, Env, and M proteins across groups and time. In addition, we observed a booster vaccination effect in these immunodominant regions with the strongest responses targeting S and Nsp3 proteins. In addition, CoV2+Vac+ individuals had broader T-cell responses than Vac+CoV2+ and showed an exclusive targeting of ORF3a, M, upORFs, Nsp2, 3C_LP, Nsp10 and Hel regions across the SARS-CoV-2 proteome. At the single peptide level, we identified differential frequency in the optimal T-cell responses across groups as the CoV2+Vac+ showed a preferential targeting of S1 (58%), S2 (17%) and ORF 1b/8 (25%) compared to Vac+CoV2+ with almost exclusive recognition of S2 (86%).

Conclusion: Our results define immunodominant long-term T-cell responses in S, Nsp3, NC, Env, and M proteins in SARS-CoV-2 proteome in the context of hybrid immunity. Our data demonstrate broader and exclusive T-cell responses in CoV2+Vac+ individuals compared to Vac+CoV2+. Overall, we identify differences in long-term T-cell hybrid immunity primed by infection
or vaccination with implications for protection from re-infection and future vaccine design.

**391 Bivalent mRNA COVID Vaccines Elicit Predominantly Cross-Reactive CD4+ T-Cell Clonotypes**

Joel Sop, Abor G. Dykema, Caroline C. Traut, Christie R. Basseth, Annukka A. Antar, Kellie N. Smith, Joel N. Blankson

The Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Background:** The emergence of the Omicron BA.5 sublineage in February 2022 raised concerns due to its ability to escape neutralizing antibodies induced by ancestral spike mRNA vaccines and natural infection with prior SARS-CoV-2 variants. Bivalent COVID-19 vaccines, containing mRNA for both ancestral and Omicron BA.5 spike proteins, were developed to enhance immune responses against the BA.5 sublineage. However, studies have shown that these bivalent vaccines do not induce stronger T cell responses to the BA.5 spike protein than monovalent vaccines containing only ancestral spike mRNA. We tested the hypothesis that this was due to the preferential expansion of cross-reactive memory T cells rather than naive BA.5 mono-reactive T cells.

**Methods:** We used the ELISPOT assay to determine the targeted epitopes and the functional expansion of specific T Cells (FEST) assay to assess the percentage of CD4+ T cells that cross-recognize both ancestral and BA.5 spike proteins compared to those that were mono-reactive for each protein. We conducted this analysis in two distinct cohorts: 20 healthy donors (HDs) and 20 people living with HIV (PLWH) on suppressive antiretroviral therapy. All the participants received either the Pfizer-BioNTech (BNT162b2) or Moderna (mRNA-1273) SARS-CoV-2 ancestral spike/BA.5 spike bivalent vaccine.

**Results:** We found robust T cell responses to both ancestral and BA.5 spike proteins in both HDs and PLWH. Importantly, the FEST assay revealed that a predominant percentage of these responses were cross-reactive CD4+ T cells. Specifically, only 8.9% and 3.8% of spike-specific CD4+ T cell receptors were mono-reactive to BA.5 spike protein in HDs and PLWH respectively. Additionally, we conducted an in-depth analysis of the individual ancestral spike protein epitopes targeted by T cells in bivalent vaccine recipients. We found that more than 80% of these epitopes were ones that did not contain BA.5 mutations.

**Conclusion:** In conclusion, our study suggests that the current bivalent vaccines do not effectively induce substantial BA.5 mono-reactive T cell responses; instead, cross-reactive T cells dominate the spike-specific T cell response. These findings have significant implications for future COVID vaccine strategies.

**392 Spike V987H Vaccination Protects Animal Models From SARS-CoV-2–Induced Severe Disease**

Carlos Ávila Nieto, Julai Vergara-Albert, Pep Ampemangui-Rigo, Erola Ainsua Enrich, Leila B. Giron, Joaquim Segalés

394 Distinct Absence of Post-Boost S-IgG Surge in Sub-Saharan African COVID-19 Vaccine Responses

Jennifer Servanga, Violet Ankunda, Joseph S. Katende, Jackson Ssembera, Gerald K. Okula, Claire Baine, Pontiano Kaleebu

Uganda Virus Research Institute, Entebbe, Uganda

**Background:** Most SARS-CoV-2 vaccines are based on a two-prolines (K986P and V987P) stabilized Spike (S) glycoprotein (S-2P). Although these mutations improve S stability and immunogenicity, S-2P is produced at low levels yet. Here we explored new S protein stabilization approaches.

**Methods:** We have investigated the immunogenicity and efficacy of a novel set of stabilizing mutations selected by computational modeling and screened by recombinant S protein and RBD exposure. S variants were produced by transient transfection in Exp293 cells, and yield and RBD exposure were analyzed by ELISA. Immunogenicity and efficacy studies were conducted in K18-hACE2 mice and golden Syrian hamsters (GSH) challenged with SARS-CoV-2 De14G, Beta or Omicron BQ.1.1 variants. We measured weight changes, and viral loads in different biological samples: oropharyngeal swab, nasal turinate, lung and brain. In addition, we performed histopathological analysis of tissue samples. Humoral and T cell responses were analyzed by ELISA and neutralizing assays, and by ELISPOT, respectively.

**Results:** When compared with the S-2P, a single V987H mutation increased RBD exposure and production, and showed equivalent immunogenicity (determined as anti-RBD, anti-S IgG levels, and IFN-g ELISPOT). S-V987H immunization induced neutralizing antibodies against Wuhan, Beta and Delta variants and gained activity against Omicron after viral challenge. S-V987H immunized mice showed lower viral loads and immunohistochemistry score than non-immunized animals and were protected from severe disease induced by SARS-CoV-2. These results were confirmed in the GSH model.

**Conclusion:** Here, we identified a novel single mutation that increased the yield of S protein, maintaining its immunogenicity and improving the protective efficacy against the development of SARS-CoV-2-induced severe disease in two animal models. These results could contribute to the development of novel vaccines for other respiratory viruses.

**393 Prior COVID-19 Alters Antibody Function in ART-Suppressed PWH Receiving SARS-CoV-2 Vaccination**

Livio Azzoni, Jan Tietjen, Qin Liu, Shalini Singh, Mansi Purwar, Matthew Fair, Paridhima Sharma, Leila B. Giron, Jianyi Ding, Linda Lalley-Characzkó, Emily Hiserodt, Karam Mounzer, David B. Weiner, Mohamed Abdel-Mohsen, Luis J. Montaner

Wistar Institute, Philadelphia, PA, USA; Philadelphia FIGHT, Philadelphia, PA, USA

**Background:** People with HIV infection (PWH) receiving suppressing antiretroviral therapy (ART) develop vaccine-driven immune responses (anti-RBD antibodies, RBD/ACE binding competition and neutralization) to early SARS-CoV-2 isolates similar to the general population. However, the impact of previous SARS-CoV-2 infection on the humoral and cellular immune response after a two-dose SARS-CoV-2 RNA vaccination in PWH on ART is unknown.

**Methods:** We collected peripheral blood from 44 PWH on ART (n=24 with prior SARS-CoV-2 infection; HIV+/COVID19 and n=20 who did not report prior SARS-CoV-2 infection; 24: HIV+/COVID19 at four time points: 1) before the 1st and 2nd dose of RNA-based vaccine, and 3 and 6 months after 2nd dose. We assessed total spike (S), RBD, and nucleocapsid (N) titers and neutralizing antibody titers (ELISA; Spike/ACE2 HTRF against Wuhan, B, & L, or o variants; pseudo-neutralization of o BA.I and BA.2, 1), Fc-mediated antibody-dependent cytotoxicity (ADCC), complement deposition (ADCD), and cellular phagocytosis (ADCP), SARS-CoV-2 and HIV-specific T cell responses (IFN-γ ELISPOT), and T cell and monocyte activation (Flow cytometry). Changes from baseline and between visits were assessed using the Wilcoxon signed rank test.

**Results:** Neutralizing antibody titers against spike protein (all variants) were elicited by vaccination in both groups, but were significantly higher and better retained in the HIV+/COVID19 group, including antibody titers against o BA.I and BA.2 pseudesotypes, compared to HIV+/.COVID19 group. Non-neutralizing antibody responses (ADCC and ADCP) were induced in both group; however, ADCD was significantly enhanced in the HIV+/COVID19 group than the HIV+/ COVID19 group. Both groups showed persistent increase in T-cell and monocyte activation following vaccination, yet without a parallel increase in T-cell-specific responses to HIV Gag or Nef.

**Conclusion:** Prior SARS-CoV-2 infection is associated with greater non-neutralizing antibody innate immune effector functions and a broader anti-SARS-CoV-2 immune response (including greater activity against Omicron variants) in PWH on ART following Wuhan strain RNA vaccination. Further studies are needed to examine the impact of vaccination-induced, Fc-mediated innate immune functions on re-infection and/or disease course.
395 Disparities in Anti-SARS-CoV-2 Reactivity According to Vaccines in the Era of Omicron in Cameroon

Ezechiel Ngoufack, Jagni Semengue, Joseph Fokam, Desire Takou, Collins Ambe Chenwi, Grace Beloumou, Joseph Fokam, Alex Durand NKA, Aurelie Minelle Njengu Kengo, Sandrine Djapra, Alexis Njiglo, Carlo Federico Perno, Vittorio Colizzi
1Centre International de Référence Chantal Biya, Yaoundé, Cameroon, 2Bambino Gesù Children’s Hospital, Rome, Italy

**Background:** Anti-SARS-CoV-2 vaccine remains a global health priority, but evidence on its significance within tropical settings like Cameroon is limited. Our objective was to assess the overall rate of COVID-19 antibodies, its disparity according to vaccine-status and types of vaccines administered in Cameroon during the active phase of Omicron variants.

**Methods:** A cross-sectional sero-survey was conducted from February throughout July-2022 (active phase of Omicron circulation) among individuals tested for COVID-19 in Yaoundé-Cameroon. Socio-demographic and detailed clinical data were collected; SARS-CoV-2 antibodies were tested on plasma using Enzyme-linked immunosorbent assay (ELISA). Anti-SARS-CoV-2 IgG and IgM antibodies were measured.

**Results:** A total of 2449 participants were enrolled: median (IQR) age was 40 (31–48), 56.4% (1382/2449) men, 2.2% (54/2449) with flu-like symptoms during the 2 weeks prior to sampling. Nearly two thirds of people living with HIV received at least 3 doses of a COVID-19 vaccine, compared with approximately half of the general Ontario population. Yet disparities in uptake remain, especially by sex; this needs further exploration due to differences in social determinants of health between males and females living with HIV. Continued monitoring of COVID-19 vaccine uptake for people living with HIV is critical to inform prevention efforts.

**Conclusion:** Continued monitoring of COVID-19 vaccine uptake for people living with HIV is critical to inform prevention efforts.

<table>
<thead>
<tr>
<th>Table 1. Adjusted risk ratios (95% confidence intervals) for uptake of ≥ 3 COVID-19 vaccine doses among people living with HIV in Ontario, Canada, as of August 31, 2022, stratified by sex.</th>
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<tr>
<td>Sex</td>
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<tr>
<td>Age (19 years)</td>
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<tr>
<td>Reference: 19–65 years</td>
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<td>Not born in Canada</td>
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<td>Reference: born in Canada</td>
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<tr>
<td>Geographic (1/3)</td>
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<tr>
<td>Alphabetically arranged by comorbidity</td>
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<td>Alphabetically arranged by comorbidity</td>
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**Notes:**

- *Model additionally adjusted for testing positive for SARS-CoV-2 prior to December 14, 2020.*
- All models were adjusted for sex, age group, geographic location, and comorbidities.

**Discussion:**

Disparities in COVID-19 vaccine uptake among people living with HIV in Ontario, Canada, as of August 31, 2022, stratified by sex and comorbidities.
COVID-19 Breakthrough Infections Among People With and Without HIV: A Statewide Cohort Analysis

Xueying Yang, Jiayia Zhang, Ziang Liu, Shujie Chen, Bankole Olatos, Gregory A. Poland, Sharon Weissman, Xiaoming Li

University of South Carolina at Columbia, Columbia, SC, USA, Mayo Clinic, Rochester, MN, USA

Background: Evidence is limited regarding the COVID-19 vaccine effectiveness among people with HIV. This study aims to characterize and compare the COVID-19 breakthrough infections between people with and without HIV across different phases of the pandemic.

Methods: Using a statewide HIV cohort data, the study population was adult residents (≥18 years old) who were fully vaccinated (i.e., receipt of the second vaccine dose of Pfizer-BioNTech or Moderna or the single-dose of the Janssen vaccine) between January 2, 2021 to April 14, 2022 when Alpha, Delta, or Omicron variant circulating in South Carolina. Vaccinated people with HIV (PWH) was matched with the vaccinated people without HIV (PWOh) via propensity score matching (PSM) with 1:2 rate. A vaccine breakthrough infection was defined as SARS-CoV-2 infection ≥14 days after fully vaccination. We used Cox proportional hazard model to investigate the association between HIV infection and breakthrough infections, adjusting for relevant covariates.

Results: Among 2,144,415 vaccinated individuals, 8,335 were PWH and 2,136,080 were PWOh. Over the 18-month observation period, the percentage of breakthrough infections among PWH and PWOh was 5.22% and 4.61% (p = 0.0084), respectively. After PSM matching, HIV infection was not significantly associated with breakthrough infection rate. However, when comparing breakthrough infections among individuals without any booster dose, PWH had a higher risk of breakthrough infections (adjusted Hazard Ratio [aHR] 1.19; 95%CI: 1.03, 1.39). Individuals who resided in a county with higher COVID-19 incidence (aHR 1.05; 95%CI: 1.02, 1.07), being vaccinated during the Omicron dominant period vs Alpha dominant period (aHR 7.02; 95%CI: 3.12, 15.80), being Black (aHR 1.20; 95%CI: 1.04, 1.39), or who had chronic pulmonary disease (aHR 1.36; 95%CI: 1.10, 1.68) were associated with higher odds of breakthrough infections, while prior COVID-19 infection (aHR 0.37; 95%CI: 0.28, 0.48) and being vaccinated with Moderna vs Pfizer (aHR 0.77; 95%CI: 0.66, 0.90) was negatively associated with the outcome. Comparing with PWOh, PWH with high levels of CD4 count or viral suppression were not associated with breakthrough infections.

Conclusion: We did not find an increased risk of breakthrough infections of PWH compared with PWOh. Receipt of a booster dose conferred further protection.


Nigel Garrett, Tarylee Reddy, Nonhlanhla Yende-Zuma, Azwhidzhi Takalani, Kate Anteyi, Kubashi Woeber, Ihsen Sechbaran, Jacqueline Ohioambo, Penny Moore, Wendy Burgers, Brett Leav, Linda-Gail Bekker, Glenda Gray, Ameena Goga, for the SHERPA Study Group

1Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, 2South African Medical Research Council, Durban, South Africa, 3Hutchinson Center Research Institute of South Africa, Cape Town, South Africa, 4Moderna, Inc, Cambridge, MA, USA, 5South African Medical Research Council, Cape Town, South Africa, 6University of the Witwatersrand, Johannesburg, South Africa, 7University of Cape Town, Cape Town, South Africa, 8 Desmond Tutu HIV Foundation, Cape Town, South Africa

Background: Given limited data on safety and effectiveness of heterologous Covid-19 vaccine boosting in lower income settings with high HIV prevalence, we evaluated an ancestral strain mRNA-1273 vaccine boost after priming with Ad26.Cov2.S in South Africa.

Methods: SHERPA was a single-arm, open-label, phase 3 study nested in the national Sisonke implementation trial of 500,000 health workers. Sisonke participants were offered mRNA-1273 boosters between May and November 2022, a period of circulating Omicron sublineages. Adverse events (AE) were self-reported. Covid-19 co-primary endpoints (1. SARS-CoV-2 infections, 2. Severe Covid-19 of hospitalizations or deaths) were identified through national databases. We used Cox regression models with mRNA-1273 booster status as a time-varying covariate to determine the relative vaccine effectiveness (rVE) of a mRNA-1273 boost among SHERPA participants compared to Sisonke participants who did not receive the booster. 200 participants contributed to an immunogenicity substudy.

Results: Of 11,248 SHERPA participants in the rVE analysis cohort (79.3% female, median age 41), 45.4% had previously received one and 54.6% two Ad26.Cov2.S vaccines. Self-reported comorbidities included HIV (18.7%), hypertension (12.9%) and diabetes (4.6%). In multivariable analysis including 413161 unboosted Sisonke participants, rVE of the booster was 59% (95%CI 29-76%) against SARS-CoV-2 infection: 77% (95%CI 9-94%) for participants with one, and 52% (95%CI 13-73%) for those with two prior Ad26.Cov2.S (Table 1). There were 148 adjudicated severe COVID-19 cases among unboosted participants, and only one among SHERPA participants, a person with severe HIV-related immunosuppression. Of 11798 SHERPA participants in the safety cohort, 271 (2.3%) reported a reactogenicity events or unsolicited AEs, more in those with prior SARS-CoV-2 infections (adjusted odds ratio (aOR) 2.03, 95%CI 1.59-2.59) and less in persons with HIV (PW) (aOR 0.49, 95%CI 0.34-0.69). No related serious AEs were reported. Antibody functions were higher 4 weeks after boosting regardless of prior Ad26.Cov2.S dosing, or HIV status. mRNA-1273 increased T- cell responses and generated spike-specific responses that were pharmacy data. Eligible children were residents of the Madrid region who were aged 6-11 years after 7 December 2021 and 12-17 years after 31 May 2021 (when vaccination was first recommended in each age group), had not been previously vaccinated, and had no prior evidence of SARS-CoV-2 infection. We emulated sequential trials by identifying eligible children who received the first dose of an mRNA vaccine each day between the start of eligibility and December 2022 and matching them (on sex, age, and postcode) with five controls who had remained unvaccinated through that day. After censoring matched sets at vaccination of an unvaccinated child, we estimated the 240-day cumulative incidence (risk) of COVID-19 hospitalization, MIS-C, and myocarditis. We used percentile-based bootstrapping to obtain 95% confidence intervals.

Results: The age group 6-11 years included 183,430 vaccinated children and 917,150 controls. Over 240 days, the estimated effectiveness (95% CI) of vaccination was 10% (-40 to 50) for COVID-19 hospitalization and 30% (-40 to 80) for MIS-C. No cases of myocarditis occurred. The age group 12-17 years included 277,758 vaccinated children and 1,388,790 controls. Over 240 days, the estimated effectiveness (95% CI) of vaccination was 50% (20 to 70) for COVID-19 hospitalization and 50% (20 to 90) for MIS-C. The risk ratio of myocarditis was 1.1 (95% CI: 0.7, 1.7) for vaccinated vs. controls.

Conclusion: In this population-based study, including over 2.8 million children, the estimated effectiveness of mRNA vaccines against COVID-19 hospitalization was modest in the age group 12-17 years and lower in the age group 6-11 years. The absolute risks of MIS-C and myocarditis were small, and under conventional statistical criteria, both increased and decreased risks after vaccination were highly compatible with the data.

Effectiveness and Safety of SARS-CoV-2 mRNA Vaccines in Children: A Population-Based Study in Madrid

Miguel Hernández, Alejandro Alvaro-Meca, Maria J. Calvo-Alcántara, Maria Luisa Navarro, Jose T. Ramos, Jose C. Estévez, Miguel Basanta, Sergio Ruiz, Angel L. Mataix, Lourdes Casanov, Aura P. Silva, Pilar Salas, Jose R. Arribas, Jose M. Molero, Juan Berengue

1Harvard TH Chan School of Public Health, Boston, MA, USA, 2Universidad Rey Juan Carlos, Madrid, Spain, 3Servicio Madrileño de Salud, Madrid, Spain, 4Hospital General Universitario Gregorio Marañón, Madrid, Spain, 5Hospital Universitario 12 de Octubre, Madrid, Spain, 6La Paz University Hospital, Madrid, Spain, 7University of South Carolina at Columbia, Columbia, SC, USA, 8University of Cape Town, Cape Town, South Africa

Background: Decisions about COVID-19 vaccination in children require an assessment of benefits such as prevention of COVID-19 hospitalization and multisystem inflammatory syndrome in children (MIS-C), as well as of risks such as myocarditis. In the absence of large, randomized trials, this evidence needs to be obtained from prospective observational studies conducted in large populations over more than 6 months.

Methods: We emulated target trials of COVID-19 vaccination among children using the population-wide databases of the Madrid Health Service, which include demographic information, primary care records, hospital data, and

Figure 1 Cumulative incidence of COVID-19 breakthrough infections stratified by HIV status and different variants of concern

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Peripheral Immune Progression to Long COVID (LC) Is Driven by Mitochondrial Gene Transcription

David P. Maison1, Vedbar Khadka1, Isam Mohd-Ibrahim1, Michael J. Peluso1, Timothy J. Henrich1, Youping Deng1, Mariana Gerschenson2
1University of California San Francisco, San Francisco, CA, USA; 2University of Hawaii, Honolulu, HI, USA

Background: Long COVID (LC) affects about 10% of persons infected with severe acute respiratory coronavirus 2 (SARS-CoV-2) and can have serious clinical consequences. Whereas the pathophysiology of LC is likely multifactorial, several hypotheses have been proposed, including viral persistence in some individuals for months following the acute phase of COVID-19. Herein, we analyzed existing human RNA transcriptome datasets from people with and without LC to identify unique phenotypic fingerprints of LC.

Methods: We analyzed 28 publicly available RNAseq datasets from COVID-19 acute and convalescent studies. Differentially expressed genes and gene ontology analysis between No known COVID (NC) (n = 448), Acute COVID (AC) (n = 694), COVID Recovered (CR) (n = 123), and LC (n = 23) were examined. All samples underwent processing using the STAR Aligner and GRCh38 reference genome. DESeq2 identified differentially expressed genes, and the most significant genes were distilled through Gene Ontology and Reactome to identify pathways. We accounted for batch effects between the studies by including ‘study’ as a covariate in the DESeq2 analysis.

Results: The cohort comparison between NC and AC revealed global changes in nucleosome assembly (p.adj = 1.23e-24), chromatin remodeling (p.adj = 0.0027), among others. Comparisons of AC to CR or LC showed a transcriptome signature enriched in mitochondrial functions.

Conclusion: RNA profiling of peripheral immune cells suggests that recovery from AC is driven by mitochondrial gene transcription. In contrast to recovery, LC correlated with electron transport chain-specific genes. These findings support our previous findings of increased oxygen consumption and ATP production in the peripheral immune cells in people with LC and further implies that host gene transcription drives this phenotype.

ERAPs Control In Vitro and Ex Vivo SARS-CoV-2 Infection by Triggering Antiviral Immune Response

Irmia Saulle, Maria Luisa Murno, Fiona Limanaqi, Micaela Garziano, Sergio Strizzi, Claudia Vanetti, Sergio Lo Caputo, Maria Cristina Poliseno, Teresa A. Santantonio, Mario Clerici, Mara Biasin
University of Milan, Milan, Italy

Background: ERAP1 and ERAP2 (ERAPs) are two endoplasmic reticulum aminopeptidases which control susceptibility/progression of different infectious diseases. Beyond their canonical role in antigen processing and presentation, following inflammatory stimuli ERAPs can be secreted and modulate both the acquired and natural immune response. We investigated in an in vitro model whether exogenous recombinant human (rh) ERAP can play a role in modulating SARS-CoV-2 infection/replication by boosting the antiviral potential of immunocompetent cells.

Methods: Granulocytes isolated from 10 healthy controls were stimulated with 300ng/mL of rhERAP1, rhERAP2 or rhERAP1+rhERAP2. After 24h, these cells were co-cultured with in vitro SARS-CoV-2 infected A549-ACE2 cell line and the following parameters were assessed: 1) viral replication; 2) neutrophil activation; 3) cytokine secretion; 4) Phagocytosis and cell migration. In parallel, ERAP1 and ERAP2 mRNA concentration were quantified in unstimulated and Spike-stimulated PBMCs as well as in plasma of 10 mild (MD) and 10 severe (SD) COVID-19 patients.

Results: Granulocytes incubation with rhERAPs significantly reduced SARS-CoV-2 replication in A549-ACE2 infected cells (p<0.05); rhERAP1+rhERAP2 (p<0.05; COMBO p<0.01). This antiviral activity was associated with: neutrophil activation and degranulation (CD15+CD16+CD66b+++MPO) (p<0.05); increased neutrophil migration (p<0.001) and reduction of SARS-CoV-2 internalized viral particles (p<0.05), and the release of several cytokines/chemokines, mainly IL-8 (p<0.01). Ex viv0 analyses showed that ERAP mRNA expression was drastically reduced in both unstimulated (p<0.05) and Spike-stimulated (p<0.05) PBMCs of SD compared to MD. Conversely, a higher ERAP expression was detected in plasma of severe MD patients (p<0.05).

Conclusion: ERAPs trigger several antimicrobial mechanisms in neutrophils suggesting that their anti-SARS-CoV-2 potential is not limited to their canonical role in Ag presentation and CBB+ T cell activation. These findings pose the premise to further investigate potential use of ERAP in novel preventive and therapeutic approaches against viral infections.

Innate Immune Response Through NOD1 Agonists Prevents SARS-CoV-2 Infection in Lung Epithelial Cells

Edurne Garcia-Vidal1, Ignasi Calba2, Eva Rivera-Muñoz2, Elisabet Garcia2, Bonaventura Clotet1, Pere Serra-Mitjà3, Cecilía Cabrera1, Ester Ballana2, Roger Badia2
1ICN32 Institute for AIDS Research, Barcelona, Spain; 2Institute for Health Science Research Germans Trias i Pujol, Badalona, Spain; 3Hospital Sant Pau, Barcelona, Spain

Background: The difficulty to exert a direct antimicrobial activity in the lung, is one of the major roadblocks for the management of respiratory infections, such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Boosting
the innate immune response of the respiratory mucosa at early stages of the infection is a potential alternative intervention for the treatment of respiratory infections.

Methods: Ability to induce innate immune response was evaluated by flow cytometry, measuring IL8 production upon 24h incubation with distinct immunomodulators targeting the main pattern receptor recognition molecules (PRRs), in lung epithelial cell AS49 or myeloid THP-1 cells. NOD-like receptor (NLR) agonists were characterized through their proinflammatory profile in AS49 and the activation of the NF-kB and the interferon- sensitive response element (ISRE) pathways in AS49-DualTM hACE2-TMPRSS2 cells (AS49-Dual). Specificity of TriDAP (NOD1) and M-TriDAP (NOD1/2) agonists in lung epithelial cells was assessed by transient downregulation of NOD1 gene expression (siRNA) and selective NOD1 and NOD1/2 inhibitors. Systemic activation by NOD agonists was evaluated in PBMCs. Antiviral activity of NOD agonists was determined in AS49-Dual cells in vitro challenged with SARS-CoV-2-GFP as measured by the percentage GFP+ by flow cytometry at 48 h infection.

Results: Amongst the immunomodulators tested, NLR agonists TriDAP (NOD1) and M-TriDAP (dual NOD1/2) showed the best potency and selectivity, inducing up to 2.5-fold increase of IL8+ cells in a dose- dependent manner, without impairing cell viability. Response to NOD1 and dual NOD1/2 agonists activity involved NF-κB and ISRE pathways induction. NOD1 downregulation (siRNA) resulted in a 93% reduction of IL8+ cells cocultured with TriDAP or M-TriDAP. Selective NOD1 and NOD1/2 inhibitors impaired the NOD1-induced activation of NF-κB and ISRE pathways. PBMCs were unresponsive to NOD1 agonists, suggesting tissue-specific activity of NOD1 in lung epithelial cells, without a global systemic activity. Finally, NOD1 agonist Tri-DAP and dual NOD1/2 agonist M-TriDAP promoted an antiviral environment that prevented SARS-CoV-2 replication in lung epithelial cells (57% protection).

Conclusion: This work provides the biological basis for the development of host-directed therapies based on the NLR pathway to boost the innate immune system for an early viral clearance and infection resolution.

403 SARS-CoV-2 Natural Infection Elicits Cross-Reactive Immunity to OC43
Micaela Garziano, Claudia Vanetti, Sergio Strizzi, Irma Saule, Maria Luisa Muro, Fiona Limanaqui, Valentina Artusa, Mario Clerici, Daria Trabattoni, Mara Biasin
University of Milan, Milan, Italy

Background: The recent SARS-CoV-2 pandemic renewed interest in other previously discovered non-severe acute respiratory syndrome human coronaviruses. Among these, OC43 is a seasonal human coronavirus widely diffused in the global population (90% seroprevalence in adults), mostly responsible for mild respiratory symptoms. As OC43 protective immunity is short lasting the aim of this study was to verify if systemic and mucosal SARS-CoV-2 humoral immunity elicited by both natural infection and/or vaccination is able to confer protection against a new OC43 re-infection.

Methods: Neutralization assay of plasma and saliva samples from 49 uninfected SARS-CoV-2-vaccinated subjects (SV), and 25 SARS-CoV-2-infected and vaccinated subjects (SV) were performed against “wild-type” SARS-CoV-2 lineage B.1 (EU) and OC43 in VeroE6 cell lines. Sampling was carried out immediately before (T0) and 15 days (T1) post third-dose administration (SV) or 15 days post-infection (SV).

Results: Neutralizing activity (NA) against SARS-CoV-2 significantly increased after third dose administration in plasma (p<0.0001) but not in saliva from SV; however, it doesn’t seem to protect against OC43. On the other hand, SARS-CoV-2 NA triggered by natural infection was able to defend against OC43 infection in both plasma (p<0.05) and saliva (p<0.01) samples.

Conclusion: Our data suggest that compared to vaccine administration, SARS-CoV-2 natural infection is able to elicit a broader and cross-reactive immunity, which results in protection from OC43 at both systemic and mucosal level. As the oral cavity represents the main entry route for coronaviruses, these results support the development of a pan-coronavirus vaccine to prevent new infections and re-infections.
moderate and severe in the full cohorts and within sex adjusted for age and BMI. A publicly available data set of bulk neutrophil sequencing was used to assess neutrophil phenotype by sex.

**Results:** Cohort 1: n=95, 48% male, median age 53, median sample 4 days after test. Cohort 2: n=59, 56% male, median age 56, median sample 3 days after test+, and on day of dexamethasone. In Cohort 1 there were 3173 DEGs in severe versus mild-moderate, enriching pathways associated with hypoxia. 54% of DEGs were shared in Cohort 1 males. Male DEGs enriched neutrophil activation/degranulation and granule pathways. In Cohort 1 females, there were few DEGs(n=111)between severe and mild-moderate; female specific DEGs enriched unfolded protein response and ER stress pathways. In Cohort 2, administration of dexamethasone ablated differential gene expression in males(n=1 DEG), but had minimal impact on DEG in females(n=149). CIBERSORT identified quantitative differences in neutrophil enrichment. Sex-stratified analysis of bulk neutrophil sequencing from 370 individuals with SARS-CoV-2 identified increased immature neutrophils in males and increased PD1+FoxP3+ neutrophils in females with severe disease. Plasma N antigen level was linked to antiviral responses, and detection was less frequent in males with severe disease.

**Conclusion:** Whole blood transcriptional responses show marked upregulation of neutrophil response genes in severe disease in males. Dexamethasone treatment ablates the transcriptional response in males with severe disease, with less impact in females. Both quantitative and qualitative differences in neutrophils contribute, and N antigen levels suggest that severe disease in males was linked to inflammation.

### 407 Regulatory T-Cell Manipulation Is Limited by Anti-Antibody Responses in HIV-1 Env-Immunized RMs

**Characterizing New and Boosted HIV-Specific T-Cell Responses Elicited by an HIV Therapeutic Vaccine**

Lily Zemelko1, Manzi Purwar1, Kara W. Chev1, Emma Reuschel1, Megan Wise1, Nicole M. Bedanov1, Laurent Humeau1, Nilu Goonetilleke1, Rafick P. Sekaly1, David B. Weiner1, Steven G. Deeks1, Rachel Rutishauser2

1University of California San Francisco, San Francisco, CA, USA, 2Wistar Institute, Philadelphia, PA, USA

Background: Regulatory T-cell (Treg) cells help mediate antigen tolerance in immune responses. We aimed to dissect the influence of anti-CD25 monoclonal antibody (mAb) on the germinal center response to immunization. We tested three mAb treatment arms: inhibiting CD25+FoxP3+CD4+ regulatory T (Treg) cells, helping mediate antigen tolerance in immune responses. We aimed to assess the effect of an HIV-1 Env immunization in immune responses.

**Methods:** Nine RMs were randomly divided into three groups that received 1 mg per infusion of Basiliximab, Anti-Tac, or CH65. Plasma antibody responses were measured at baseline, 2 weeks after the last vaccine (Week [Wk] 14), and at Wk48. Responses were defined as “new” at Wk14 if they were absent at BL with a fold change (FC)≥2, and “boosted” if present at BL and Wk14 with FC≥2. We then performed a 6-day in vitro peptide stimulation assay with Cell Trace Violet (CTV) to discern CD4+/CD8+ identity and quantify the proliferative capacity of the responses (%CVTlo of total CD4+ or CD8+ T cells).

**Results:** At Wk14 versus BL, 10 new Gag-specific T cell responses formed in 5 vaccinated participants (4 in GP arm, 1 in GPE) and 26 Gag-specific T cell responses were boosted in 8 vaccinated participants (4 in GP, 4 in GPE, Fig 1A); there were no new or boosted responses in the placebo group. CD8+ T cells responded to 4 of the new and 20 of the boosted peptides. New responses had a median (IQR) magnitude of 48 (36-55) SFU/10^6 PBMC and proliferation of 1.89% (1.13-4.43%). Between BL and Wk14, boosted response magnitude increased from 23 (17-70) to 134 (60-179) SFU/10^6 PBMC (p=0.02 boosted vs new at Wk14). Proliferation of boosted responses was unchanged (0.70% [0.41-1.00%] at BL, 0.91% [0.39-2.27%] at Wk14). At Wk48, 5 of the new responses remained detectable, and 11 of the boosted responses remained ≥2 fold above BL magnitude (Fig 1B).

**Conclusion:** HIV DNA therapeutic vaccination both boosted pre-existing T cell responses and also elicited responses against new epitopes. The higher magnitude of boosted responses by IFNγ ELISPOT post-vaccination yet similar proliferative responses suggest their per-cell proliferative capacity is lower than that of new responses. Eliciting new T cell responses may be required in order for HIV therapeutic vaccines to enhance T cell function.
408  Safety of Therapeutic HIV-1 Vaccine for Adolescents with Early Treated Perinatal HIV Infection
Shaun L. Barnaba1, Mark F. Cotton1, Nicola Cotugno2, Britta Wahren3, Pontus Blomberg3, Ellen Turk4, Mark S. de Souza5, Els Dobbel5, Vasymeel Akhalwaya5, Samantha Fry1, Hans Spiegel6, Patrick Jean-Philippe7, Paolo Palmaz2, Merlin L. Rubbi, for the Hurricane Study Team

Stellenbosch University, Cape Town, South Africa; Karolinska Institute, Stockholm, Sweden; Henry M Jackson Foundation, Rockville, MD, USA; Institute for HIV Research, Essen, Germany; 'Henry M Jackson Foundation, Bethesda, MD, USA, 'National Institute of Allergy and Infectious Diseases, Rockville, MD, USA

Background: HVRCARE is a phase I, proof of concept, open-label, randomized clinical trial to evaluate safety, immunogenicity and efficacy in HIV reservoir reduction of a prime-boost strategy with a multigene, multi-subtype A, B, C HIV-DNA vaccine (HIVWS DNA) and modified vaccinia Ankara Chiang Mai Double Recombinant (MVA-CMDR) vaccine ± co-administration of Toll-like Receptor 4 agonist (within Cervarix® human papilloma vaccine) in adolescents and youth living with perinatally acquired HIV-1.

Methods: Twenty-five South African adolescents living with perinatal HIV, 14 to 16 years of age were enrolled. All started antiretroviral therapy prior to 6 months of age, with continuous viral suppression through enrollment, and randomized to HIV vaccines only (n=10, Arm 1), HIV vaccines and Cervarix® (n=10, Arm 2) or Cervarix® only (n=5, Arm 3). The HIV DNA vaccines were administered through a needle free device (PharmaJet®) at weeks 0 and 4 ± Cervarix® and the MVA-CMDR at 24 (± Cervarix®) and 36 weeks (MVA-CMDR only). Local and systemic reactions were captured 30 minutes after vaccination and on diary cards for seven days. Pregnancy was screened at each visit.

Results: Two participants missed week 24 immunization (due to TB and pregnancy). There were no loss to follow up, no deaths and 15 participants have completed their week 60 visits. Local and systemic reactogenicity reported as mild or moderate in all 25 participants: 24 (96%) had local and 21 (84%) systemic reactions. Five participants with severe reactogenicity all reduced to moderate, mild or resolved within 2 days. There was one serious adverse event (SAE) and 2 adverse events (AEs) above Grade 2, all in Arm 2. The SAE (acute appendicitis) was unrelated to study participation. One Grade 3 AE (reduced estimated glomerular filtration rate) was considered tenofovir- and not vaccine-related. The other Grade 3 AE was headache for 10 days that was severe on days 1 and 2. A pregnancy during the study was noted (Arm 2) and was electively terminated. The first MVA-CMDR vaccine was omitted due to the pregnancy, but the final CMDR was given after termination. No vaccine related AE prompted discontinuation of investigational product. All participants are expected to complete the study by mid-October 2023.

Conclusion: This study represents the first combined therapeutic HIV vaccine study in pediatrics. While all participants reported local and/or systemic reactions to vaccination, most events were self-limiting and no dose limiting AEs were reported.

Table 1: Severe reaction after vaccination

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409  WITHDRAWN

410  Linking TCR αβ Chains to Costimulatory Signaling Domains Enhances HIV-Specific CD8 T-Cell Function
Marta Santos Bravo, Harris Goldstein
Albert Einstein College of Medicine, Bronx, NY, USA

Background: The addition of costimulatory signalling domains to the CAR construct has dramatically increased the functional capacity of CAR-T cells to kill cancer cells. We hypothesized that we could augment the functional anti-HIV activity and persistence of cytotoxic CD8 T cells (CTLs) engineered to express HIV-specific TCRs used as an adoptive therapy to provide a functional HIV cure by incorporating the intracellular activation domain of costimulatory signalling domains into the TCR construct, termed TCR-costim.

Methods: As a proof-of-concept, we constructed a lentiviral vector (LV) encoding chimeric α and β TCR chains containing the well described HIV-1 Gag epitope SL9 human variable TCR region fused to the murine constant region, to prevent TCR mispairing with the endogenous TCR. We added the signal transduction domain of either the CD28 or 41BB to the α and/or β TCR chains. Jurkat/MA-NFAT-luciferase reporter (Jurma) cells were transduced with the LV to evaluate SL9-TCR activation by quantification of the luciferase reporter. SL9-TCR expression in CTLs from seronegative donors was determined by tetramer staining; and their cytotoxic activity, activation, and IFNγ and TNFα production by flow cytometry.

Results: In Jurma cells transduced with the different SL9-TCR LV constructs, TCR was highly expressed (95-99%). Jurkat/MA transduced with the SL9-CD28 LV displayed 1.47 or 2.2-fold greater TCR activation than SL9-TCR or SL9-41BB, respectively, after co-culturing with SL9-loaded T2 cells. Donor D8 T cells were effectively transduced (15-30%) with all LV constructs and SL9-TCR expression reached 80% of the transduced cells. SL9 reactivity was demonstrated by increased LAG3 and CD25 expression in SL9-TCR, SL9-CD28 and SL9-41BB CTLs incubated with SL9-loaded T2 cells but not with T2 cells alone. Of great interest was the increased cytotoxicity (figure 1A) and IFNγ and TNFα production of SL9-CD28 and SL9-41BB CTLs as compared with SL9-TCR CTLs (figure 1B).

Conclusion: Incorporating costimulatory signalling domains with the TCR construct (TCR-costim) provides an increased downstream activation of the TCR and an improved functional activity of CTLs. Because TCRs require recognition of far fewer HIV peptides than CARs to activate their effector functions, adoptive therapy with TCR-costim CTLs may overcome CAR-T cell limitations in recognizing and killing latent HIV infected cells which produce low levels of HIV proteins, and provide the sustained CTLs response required for a functional HIV cure.

Figure 1A. SL9-TCR-costim T cells showed an enhanced cytotoxic activity when T2 cells presented SL9 peptide respect to no peptide. B. Increased production of IFNγ and TNFα was detected in SL9-TCR-costim T cells after 5 hours stimulation with SL9-loaded T2 cells by intracellular staining.

411 ABBV-382, an Anti-α4β7 Ab That Enhances HIV-1 Antigen Presentation for Immune-Mediated Viral Control
Teresa Ng, Gautam K. Sahu, Domenick E. Kennedy, Tatyana Dekhtyar, Renee Miller, Liangjun Lu, Dolonchampa Maji, Silvino Sousa, Keenan Taylor, Sahana Bose, Joel F. Cohen-Solal, Axel Hernandez Jr., Victoria A. Pitney, Melanie J. Patterson, Jochen Salfeld
Abbvie, Inc, Chicago, IL, USA

Background: The α4β7 integrin plays an important role in the pathogenesis of HIV-1 infection. It is a heterodimeric receptor expressed on different immune cells. Expression of α4β7 on peripheral CD4+ T cells predicts HIV-1 acquisition and disease progression. Recent study showed that α4β7 is present on the envelope of HIV-1 virions, which suggests that α4β7 could be a highly conserved target for HIV-1 intervention.

Methods: ABBV-382, a humanized mouse anti-human α4β7 mAb, has been evaluated in different biochemical, virological, immunosafety, and immunopeptidomics studies to characterize its properties and determine its mechanisms of action for HIV-1 intervention.

Results: ABBV-382 demonstrated high binding affinity to α4β7 and blocked the interaction of α4β7 with its ligand MAdCAM-1. The binding profile of ABBV-382 to human FcγRs was similar to that of a typical human IgG1 mAb, but it did not induce ADCC or ADCP activity in vitro. ABBV-382 blocked the MAdCAM-1- mediated co-stimulation of CD4+ T cells, and HIV-1 replication in these treated cells. It also inhibited the interaction of α4β7 with HIV-1 gp120, and is therefore proposed to inhibit the cell-to-cell viral spread mediated by
this interaction. Consistent with the report that α4β7 is present on the surface of HIV-1 virions, ABBV-382 could bind to virions from different HIV-1 strains to form immune complexes (ICs). These ICs could engage different FcγRs through the Fc domain of ABBV-382 in vitro. When the ICs were incubated with antigen presenting cells (APCs), they were phagocyctosed in a Fc-dependent manner. Immunoproteomics analyses demonstrated that the internalized ICs were processed, and HIV-1 peptides were processed by MHC class II on APCs, a mechanism that is proposed to enhance HIV-1 antigen presentation to T cells. 

Conclusion: Our study results provide evidence of the antiviral and immunomodulatory properties of ABBV-382 through two main mechanisms: (1) Direct antagonism of the interaction of α4β7 with its ligand MadCAM-1 or HIV-1 gp120, leading to inhibition of HIV-1 replication or cell- to-cell viral spread, respectively, and (2) Enhanced viral antigen presentation to T cells enabled by the Fc-dependent uptake of ICs (HIV-1 virions and ABBV-382) by APCs. Taken together, ABBV-382 demonstrates favorable biological characteristics and novel mechanistic properties supporting its clinical evaluation as an immune-based intervention for HIV-1 viral control. Disclosure: The design, study conduct, & financial support were provided by AbbVie.

412 10e8.4/iMAb Bispecific Antibody for Immunoprophylaxis Against High-Dose Intravenous SHIV Exposure
Matthew S. Parsons1, Hannah A. King1, Decha Silisorn1, Jumplomp Sopanaporn1, Panuput Nadee1, Dutsadet Inthawong1, Raviviran Immerbin1, Caroline Subra1, Lindsay Wizenczuk2, Victoria Polonis1, Yaoxing Huang1, David D. Ho1, Sandhya Vasani1, Julie Ake1, Diane L. Bolton1
1 Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, 2US Military HIV Research Program, Silver Spring, MD, USA, 3Aaron Diamond AIDS Research Center, New York, NY, USA

Background: A lead strategy for preventing HIV transmission is the passive provision of broadly neutralizing antibodies (bNAbs). Various investigators have validated this approach in in vitro and in vivo (NHP) models of HIV exposure. Experiments in NHPs have primarily focused on preventing viral transmission across anogenital mucosa. In humans, the antibody-mediated neutralization (AMP) trial demonstrated that passively administered VRC01 bNAb prevents the transmission of neutralization-sensitive but not resistant HIV strains, emphasizing the urgent need for bNAbs with greater breadth and potency for immunoprophylaxis strategies.

Methods: We developed a nonhuman primate model of immunoprophylaxis in the context of high-dose intravenous HIV exposure. We assessed the 10e8.4/iMAb bispecific antibody, which targets the membrane- proximal external region of the HIV envelope and the CD4 receptor, as an immunoprophylaxis agent. We measured in the vitro neutralization activity of 10e8.4/iMAb using the TZM-bl cell line-based neutralization assay. We intravenously infused three animals with 30mg/kg of 10e8.4/iMAb and three control animals with PBS. One hour later, we challenged all animals intravenously with a high dose (~50,000 TCID50) of SHIV-BG505. We performed antibody-mediated CD8+ lymphocyte depletions eight weeks after the viral challenge to assess viral transmission across anogenital mucosa. In humans, the antibody-mediated neutralization (AMP) trial demonstrated that passively administered VRC01 bNAb prevents the transmission of neutralization-sensitive but not resistant HIV strains, emphasizing the urgent need for bNAbs with greater breadth and potency for immunoprophylaxis strategies.

Results: The SHIV-BG505 challenge virus was sensitive to 10e8.4/iMAb (IC50 <0.01 ug/ml). Control animals developed plasma viremia one week after high-dose intravenous viral challenge (peak: 8.7E6 – 1.1E7 copies/ml). Animals passively administered 10e8.4/iMAb exhibited no evidence of viral infection, even five weeks after systemic depletion of CD8+ lymphocytes.

Conclusion: Passive intravenous provision of potent anti-HIV bispecific bNAbs is a highly promising immunoprophylaxis strategy for high-dose intravenous HIV exposure.

413 Long Half-Life Broadly Neutralizing Killer Bispecifics Against HIV-1: Harnessing the Immune System
Sukanya Ghosh, Maya Singh, Mansi Purwar, Daniel Kulp, Luis J. Montaner, David B. Weiner
Wistar Institute, Philadelphia, PA, USA

Background: HIV-1 remains a significant global health challenge, requiring continuous efforts to develop innovative therapeutic approaches. Clinical use of mAb needs multiple infusions and for BiTEs is limited by short half-life in blood, demanding continuous infusion resulting in high costs inconvenience and time- consumption. We describe the development of DNA-launched, bispecific T cell engagers that redirect T cells towards killing target HIV infected cells. These are highly specific Broadly neutralizing Killers (BnKs) that demonstrate long half-lives (LHL) in vivo.

Methods: We designed DNA-launched LHL- BnKs with bispecific scFvs and a Fc fragment having Fc effector- null mutations to abrogate Fcy receptor binding. We evaluated in- vitro and in- vivo expression of DNA launched LHL-BnKs and characterized functionality. ELISA and flow cytometry were used to demonstrate binding studies, pseudo-neutralization assays to test neutralizing potential against a global virus panel. A novel cell based Xcellence assay was designed which uses impedance as a readout for killing activity. In- vivo pharmacokinetics were evaluated by inoculating the DNA launched LHL-BnKs in BALB/c mice.

Results: DNA launched PGDM1400, 3BNC117 and PGT121 LHL-BnKs respectively were designed and optimized for expression both in- vitro and in- vivo. Binding studies demonstrate that these molecules bind to CD3 in a concentration dependent manner. They also demonstrated binding to CD3+ T cells and neutralized a global panel of HIV-1 Tier 2/3 viruses with high potency and specificity. The LHL-BnKs displayed potent killing of HIV-1 infected target cells with nanogram/ml IC50 for killing. Combination therapy of 10-1074 and 3BNC117 did significantly reduce viral rebound in clinical trials where the groups received up to 8 infusions of 3BNC117 during the 24-week period. Our data suggests that after single inoculation of 50ug DNA, plasma concentration of ~10ug/ml was achieved in vivo with peak serum expression around day 21 and expressed for extended duration.

Conclusion: Our data highlights the design and characterization of novel DNA launched immuno-therapeutic against HIV-1, which possess significant characteristics of a promising immunotherapy having broad neutralization capacities of a Tier 2/3 global virus panel, specific engagement of effector cells and killing of the infected target cells and longer serum half-life in comparison to conventional bispecific T cell engaging molecules. Delivery of LHL-BnKs as combination therapy.

414 Targeting HIV-Infected Cells for Immunotherapy Through HLA-E
Niklas Bachmann, Srona Sengupta, Robert F. Siliciano
The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Cytolytic T lymphocytes (CTLs) help control viremia in people with HIV. HIV evades this response through escape mutations in CTL epitopes and downregulation of HLA molecules. The dimorphic non-canonical HLA-E molecules, however, are upregulated during infection, making them promising targets for CTL-engaging reagents such as single-chain diabodies (scDbs) provided that they present HIV epitopes. Here, we show that HIV-infected cells can be targeted for CTL-mediated lysis using novel scDbs specific for HLA-E bound epitopes.

Methods: To validate HLA-E as a suitable scDb target, we used the previously characterized M. tuberculosis- derived peptide MBt44 that binds with high affinity to HLA-E. Through phage biopanning, we isolated a novel scDb (RPLP13) that recognizes this epitope bound to HLA-E. This scDb also recognizes CD3 and is able to induce CTL activation. The specificity of RPLP13 was tested through killing assays with CD8+ effector T cells and target cells presenting cognate MBt44 peptide or the irrelevant canonical HLA-E epitope VLP9. To test whether HIV- encoded peptides can be targeted, an MBt44-encoding DNA segment was inserted into an HIV reporter virus (NL4.3.EαM-GFP). Target cells were infected with this virus and co-cultured with RLP13 scDb and autologous CTLs to assess killing of infected cells.

Results: RLP13 scDb induced antigen-specific lysis of MBt44-presenting target cells, regardless of the donor HLA type, in an scDb- and antigen- dose-dependent manner. Furthermore, addition of the RPLP13 scDb to co- cultures of primary CD8+ T cells and autologous CD4+ T cells infected with viral reporter constructs carrying the MBt44 epitope led to a 50% reduction of the HIV population, indicating that targeting of HIV-1 peptides presented on HLA-E can potentially lead to the elimination of infected cells.

Conclusion: Our findings with scDb RPLP13 demonstrate that virally encoded peptides presented in the context of HLA-E can be targeted by scDbs to induce CTL-mediated lysis of infected cells, regardless of HLA-genotype. The development of novel scDbs recognizing HIV-derived epitopes that bind HLA-E would provide a universal reagent that could be used to promote the killing of HIV-1 infected cells in shock and kill strategies for HIV cure.
415 **Instability of the HLA-E Peptidome of HIV Presents a Major Barrier to Therapeutic Targeting**


Immunocore, Abingdon, United Kingdom

**Background:** Human leukocyte antigen (HLA) class Ia molecules present peptides derived from the ER for recognition by cytotoxic T lymphocytes. As such, HLA class Ia-restricted peptides are well characterised drug targets for infectious diseases. However, their clinical potential is limited by the high polymorphism of the HLA class I genes within the population. In contrast, the HLA class Ib molecule, HLA-E, has only two alleles and thus offers the potential to develop donor-unrestricted therapies. With only a few reported HIV-derived HLA-E restricted peptides, there is limited understanding of the composition and druggability of the HIV HLA-E ligandome. We therefore sought to systematically interrogate the HIV HLA-E ligandome to identify stably presented peptides as drug targets for a potential HIV cure strategy.

**Methods:** The HIV proteome of in vitro infected cells was interrogated for HLA-E presented peptides using both immunopeptidomic (mass spectrometry) and bioinformatic (predictive) approaches. Putative ligands were then ranked based on stability using a panel of biochemical assays as well as an HLA-E cell surface stabilization assay. The stability of the highest ranked peptide, Gag275-283, was further assessed using an affinity-enhanced T cell receptor bispecific molecule (ImmTAX). The ImmTAX was used as a probe for peptide-HLA-E complexes on antigen-presenting cells (peptide-pulsed targets or HIV-infected cells) in T cell redirected assays.

**Results:** Tandem mass spectrometry analysis of 1 billion infected C8166 cells (19.4% Gag+ by intracellular staining) failed to identify any HIV HLA-E peptides. Of >80 bioinformatically predicted ligands, all were characterised by low or unmeasurable complex thermostability and half-lives and performed poorly in the cell surface stabilization assay. In T cell redirected assays using the ImmTAX, consistent presentation of Gag275-283 by HLA-E was only demonstrated when peptide was supplied exogenously (peptide pulsing model). Only sporadic and inconsistent peptide presentation was detected when the peptide source was endogenously-expressed HIV Gag protein (infected cells).

**Conclusion:** This study provides biochemical and biological evidence for the failure of HIV-derived peptides to form a stable complex with HLA-E, highlighting a major challenge for HLA-E targeted drug or vaccine development.

416 **Immune Responses Associated With Mpxo Viral Clearance in People With and Without HIV Infection**

Igor Moraes-Cardoso, Susana Benet, Julieta Carabelli, Daniel Perez-Zsolt, Adrià Mendoza, Vicente Descazlo, Yuvennine Alarcón-Soto, Alba Grifoni, Michael Marks, Nuria Izquierdo-Useros, Jorge Carrillo, Carolina Scagnolari, Vicente Descalzo, Jorge Carrillo, Federica Frasca

1. Instituto de Investigación Sanitaria La Jolla, La Jolla, CA, USA
2. University of Pisa, Pisa, Italy
3. Università Cattolica del Sacro Cuore, Rome, Italy
4. National Taiwan University Hospital, Taipei, Taiwan
5. La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

**Background:** During the emergence of the global mpxo outbreak in May 2022, over 90,000 cases were diagnosed, disproportionately affecting those living with HIV. Milder re-infections have been recently reported. Here, we extensively characterized cellular and humoral responses in people without HIV (PWoH) and with HIV (PWH) at acute mpxo, determined their impact on disease severity and viral clearance dynamics, and assessed post-infection immunity 6 months after mpxo virus (MPXV) diagnosis.

**Methods:** Thirty-three men (PWoH n=19; PWH n=14, all with CD4 T cell>400) from a prospective, observational cohort study (NCT03547674) were included. Samples from skin lesions were collected weekly to estimate the time to clear MPXV. Blood samples were taken at diagnosis and 29, 91, and 182 days later for immune analysis. IgG and IgA against A3SL, H3R and A29L MPXV proteins were quantified by ELISA, and in-vivo neutralization capacity was measured in Vero E6 cells. T-cell responses were characterized upon antigen recall by IFNγ ELispot and multiparametric flow cytometry.

**Results:** PWoH and PWH had similar clinical severity and time to MPXV clearance in skin lesions. Both PWoH and PWH had comparable levels of anti-MPXV IgG and broad IgA-mediated responses. Whilst in-vivo neutralization was not fully observed until 91 days after infection, both titers and breadth of antibodies induced early after infection were associated with reduced clinical severity, lower levels of virus in skin lesions and a shorter and more rapid viral clearance. Levels of antibodies increased one month after MPXV infection and waned thereafter, while frequencies of mpxo-specific T-cells were sustained up to 6 months, regardless of HIV status. Although no major differences were observed in cellular activation or cytokine production between study groups, a delay in the contribution of multifunctional CD4+ T-cells was only observed in PWH. Overall, GzmB+ CD4+ and CD8+ T-cells were the predominant subsets contributing across all timepoints in both groups.

**Conclusion:** Although PWoH and PWH had comparable immune responses at acute mpxo, a delay on functional T-cell diversity and a faster wane of antibodies was observed in PWH. Mpxo-specific antibodies may play a key role in the initial control of infection via non-neutralizing pathways, and a durable cellular immunity is induced following infection, which might help to contain viral spread, contribute to a faster clearance, and reduce severity in future re-infections.

417 **Orthopoxvirus-Specific IgG Upon Mpxo Vaccination Among People With and Those Without HIV**


National Taiwan University Hospital, Taipei, Taiwan

**Background:** In Taiwan, nationwide routine and compulsory smallpox vaccination with Lister strain had been stopped in 1979. As mpxo continued to spread worldwide, a two-dose Mpxo vaccination, with an interval of at least 28 days, is currently recommended as pre- and post-exposure prophylaxis in the high-risk populations. However, orthopoxvirus-specific (OPXV) IgG remains less investigated in people with HIV (PWH), especially for those born after 1979.

**Methods:** Men who have sex with men (MSM) planning to receive two doses of MVA-BN vaccine were enrolled. Blood samples were collected on D0, 28±7, and 96±7 for all individuals after each dose. All serum specimens were tested for the presence of OPXV-specific IgG. Those testing positive for OPXV-specific IgG and being infected with Mpxo were excluded from subsequent analysis.

**Results:** A total of 299 participants, including 188 PWoH and 111 people without HIV (PWH), were included. The median age of the participants for PWH and PWoH was 38 and 31 years, respectively; 51 PWH and 3 PWoH were born before 1979. Most of the included PWH (97.9%) were virologically suppressed with antiretroviral therapy and had a median CD4 count of 648 cells/μL.

Overall, 16 (8.5%) of 188 PWoH and 8 (7.2%) of 111 PWH had seroconversion of OPXV-specific IgG 28 days after the first dose, while 31 (24.8%) of 125 PWH and 12 (19.4%) of 62 PWoH had a seroconversion 28 days after the second dose of Mpxo vaccination. The median titer of OPXV-specific IgG for PWH and PWoH was 3.8 and 6.4 ng/mL, respectively. Those born before 1979 were more likely to seroconvert after Mpxo vaccination than those born after 1979 (51.3% vs 16%; adjusted odds ratio [aOR], 5.54; 95% CI, 2.54-12.07). After excluding PWH born before 1979, PWH tended to have a lower seroconversion rate than PWoH (14.6% vs 18.0%; aOR, 0.75; 95% CI, 0.32-1.87).

**Conclusion:** The median OPXV-specific IgG titer among individuals who received two doses of Mpxo vaccine was higher in those who had had prior smallpox vaccination. For those born after 1979, OPXV-specific IgG was higher in PWH compared to virologically-suppressed PWH, though the differences between the two groups did not reach statistical significance, probably related to a relatively small case number.

418 **MPXV Replication Induces an IFN Response and Is Suppressed by IFN-γ**

Alessandra D’Auria, Federica Frasca, Lucia Bordi, Eleonora Lalli, Matteo Fracella, Leonardo Sorrentino, Gabriella D’Ettorre, Gaudio Maria Mastromarino, Mauro Pistello, Guido Antonacci, Carolina Scagnolari

1. Sapienza University of Rome, Rome, Italy
2. University of Pisa, Pisa, Italy
3. University of Rome, Rome, Italy
4. Istituto Superiore di Sanità, Rome, Italy
5. University of Pisa, Pisa, Italy

**Background:** Orthopoxviruses have developed strategies to evade host antiviral immunity, particularly the Interferon (IFN) response, but limited data are available for Monkeypox virus (MPXV). To better understand how MPXV affects the IFN response, we analyzed type I, II and III IFNs and IFN stimulated genes (ISGs) expression in different MPXV-infected anatomical sites from male patients. Also, we investigate MPXV in vitro sensitivity to different type of IFNs.

**Methods:** Eighteen samples from different anatomical sites (Skin lesions, SL) and 30 (19.4% Gag+ by intracellular staining) failed to identify any HIV HLA-E peptides. Of >80 bioinformatically predicted ligands, all were characterised by low or unmeasurable complex thermostability and half-lives and performed poorly in the cell surface stabilization assay. In T cell redirected assays using the ImmTAX, consistent presentation of Gag275-283 by HLA-E was only demonstrated when peptide was supplied exogenously (peptide pulsing model). Only sporadic and inconsistent peptide presentation was detected when the peptide source was endogenously-expressed HIV Gag protein (infected cells).

**Conclusion:** This study provides biochemical and biological evidence for the failure of HIV-derived peptides to form a stable complex with HLA-E, highlighting a major challenge for HLA-E targeted drug or vaccine development.

**Results:** Tandem mass spectrometry analysis of 1 billion infected C8166 cells (19.4% Gag+ by intracellular staining) failed to identify any HIV HLA-E peptides. Of >80 bioinformatically predicted ligands, all were characterised by low or unmeasurable complex thermostability and half-lives and performed poorly in the cell surface stabilization assay. In T cell redirected assays using the ImmTAX, consistent presentation of Gag275-283 by HLA-E was only demonstrated when peptide was supplied exogenously (peptide pulsing model). Only sporadic and inconsistent peptide presentation was detected when the peptide source was endogenously-expressed HIV Gag protein (infected cells).

**Conclusion:** This study provides biochemical and biological evidence for the failure of HIV-derived peptides to form a stable complex with HLA-E, highlighting a major challenge for HLA-E targeted drug or vaccine development.
measured by quantitative RT-PCR assays. MPXV-DNA levels were evaluated by Bioperfectus Monkeypox Virus Real Time PCR kit. Antiviral assays were performed to evaluate MPXV (MOI=0.1) sensitivity to different types of IFNs (IFN-α, IFN-α natural, IFN-β, IFN-ω and IFN-γ) on different cell lines (human lung adenocarcinoma epithelial A549 cells, cervical carcinoma HeLa cells and african green monkey kidney VeroE6 cells). Viral yield after IFN treatment was quantified in VeroE6 cells.

**Results:** Examination of IFN-related genes across anatomical sites revealed that IFN-α, IFN-ω, and PKR were significantly upregulated in A549 compared with other clinical samples. Conversely, levels of IFN-ω and IFN-α increased in S (p<0.05). IFN-1/II and ISGs showed higher expression in samples with low MPXV-DNA Ct values, while the opposite trend was observed for IFN-γ production (p<0.05 for all the genes). Pre-treatment of A549, HeLa, and VeroE6 cells with high concentrations of IFN-α, IFN-ω, and IFN-α (greater than or equal to 300,000 IU/mL) did not significantly inhibit MPXV replication, while MPXV exhibited moderate sensitivity to the antiviral action of IFN-β. In contrast, IFN-γ strongly inhibited MPXV replication in all the cell lines used.

**Conclusion:** This study provides evidence that MPXV infection triggers the production of various types of IFNs and ISGs, with IFN-γ being particularly effective in inhibiting MPXV replication in vitro. These findings contribute to a better understanding of how MPXV evades the host immune response and may inform potential therapeutic strategies for MPXV infections.

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419 Early Tecovirimat Treatment for Mppox Disease Among People With HIV: A Matched Cohort Analysis

Bruce Aldred, Robert H. Lyles, Jane Y. Scott, Daniel J. Gromer, Almada Aldredge, Kimberly Workowski, Boguehm K. Titi, Minh Nguyen, Vincent Marconi, Colleen Kelley, Jesse T. Jacob, Jonathan Colasanti, Emily J. Cartwright, Valeria D. Cantos, for the Emory Mpox Analysis Patient Series (MAPS) Study Group

**Background:** Despite a lack of efficacy data in humans, tecovirimat was widely prescribed to people with HIV (PWH) with mppox during the 2022 mppox epidemic, particularly among those with low CD4+ T-cell counts or severe mppox clinical manifestations.

**Methods:** This is a matched cohort study of PWH diagnosed with mppox at four hospitals in Atlanta, Georgia between 6/1/2022 and 10/7/2022. The exposure cohort (“early tecovirimat”) included PWH with mppox who were treated with tecovirimat within 7 days of symptom onset. The unexposed cohort (“late/no tecovirimat”) included PWH diagnosed with mppox who did not receive tecovirimat or who received tecovirimat >7 days after symptom onset. Multivariate logistic regression models were used to identify factors associated with progression of mppox disease, defined as development of at least one severe mppox criterion after symptom day 7. The 2 cohorts were matched 1:1 using propensity scores based on the identified predictors.

**Results:** Each cohort included 56 matched individuals. Predictors selected for inclusion in the final propensity score estimation model included age, race, HIV viral load suppression, involvement of any mucosal site(s), and hospitalization at day 7. Mppox progression occurred in 3 (5.4%) individuals in the early tecovirimat cohort and in 13 (23.2%) individuals in the late/no tecovirimat cohort (paired odds ratio 11.0 (95% CI 1.4, 85.1)), exact binomial test p = 0.006.

**Conclusion:** PWH with mppox who were prescribed tecovirimat within 7 days of symptom onset were less likely to have mppox progression compared with matched PWH who did not receive early tecovirimat. While awaiting the completion of randomized controlled trials of tecovirimat efficacy for mppox, these results favor starting tecovirimat in all PWH as soon as a mppox diagnosis is suspected.

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420 Combination of Extended Antivirals With Antiretrovirals for Severe Mppox in Advanced HIV Infection

Michael T. Duong, Pablo Tebas, Bhavya Ancha, Jillian Baron, Stuart N. Isaacs, Zsofia Szep

**University of Pennsylvania, Philadelphia, PA, USA**

**Background:** Appropriate therapeutic management of mppox in people with advanced HIV remains challenging given an absence of clinical trial evidence. This case series aims to provide our experience during the 2022 outbreak for patients with severe mppox treated aggressively with a combination of extended tecovirimat and cidofovir with antiretrovirals (ART).

**Methods:** This is a retrospective review of cases at the University of Pennsylvania. The results of December 2022 mppox outbreak in Philadelphia, PA, USA.

**Results:** We identified four male patients with mppox and advanced HIV (CD4 count 0-53 cells/mm³, viral load 2,104-217,000 copies/mL, age range 30s-50s). They exhibited nectric skin lesions across the face, trunk, groin and extremities, with one development keratits and conjunctival ulcers. Three cases were associated with super- and co-infections: HSV1/2, severe COVID-19 and MRSA. All patients started/resumed ART and began daily oral tecovirimat and cidofovir infusions with probenecid/fluids every 2 weeks. While one patient exhibited full recovery after a single 6-week course of tecovirimat, 6 doses of cidofovir and ART, the other three had a more complicated course including two with insufficient daily nutrition affecting oral tecovirimat absorption and another lacking consistent housing and wound care that led to recurrent presentations that required extended tecovirimat (5-16 weeks) and cidofovir (1-12 doses). We stopped cidofovir after 7 months on one patient based on IgG+/IgM– orthopoxvirus serology. Oral lesions were treated with trifluridine (3 weeks). Two patients required adjustments of ART. After ART, all cases had markedly lower HIV load (0-138 copies/mL). All patients had improved mppox lesions at follow-up and renal function remained stable.

**Conclusion:** This report illustrates that prolonged cidofovir with tecovirimat can be safely administered to manage severe mppox infections in people with advanced HIV. Our case series underscores the multifaceted challenges this population faces, extending beyond clinical manifestations, to include social determinants such as housing stability, nutritional intake essential for effective tecovirimat absorption and presence of concurrent infections. Our findings advocate for a comprehensive approach integrating ART with multiple antivirals, while simultaneously addressing contextual and socio-environmental factors.

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**Table:** Summary of clinical cases with mppox and advanced HIV at our institution.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>&lt;15</th>
<th>15-18</th>
<th>19-25</th>
<th>26-40</th>
<th>41+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>25</td>
<td>12</td>
<td>32</td>
<td>25</td>
<td>25</td>
<td>125</td>
</tr>
<tr>
<td>Black</td>
<td>25</td>
<td>12</td>
<td>32</td>
<td>25</td>
<td>25</td>
<td>125</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Asian</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>20</td>
</tr>
</tbody>
</table>

**Table:** Matched Cohort: Courtesies and Outcome

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Median (QR) or N (%)</th>
<th>Tecovirimat or/and before symptom day 7</th>
<th>No hemorrhea or/and before symptom day 7</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>0.025</td>
</tr>
<tr>
<td>Race</td>
<td>Black</td>
<td>25 (8:25)</td>
<td>25 (8:25)</td>
<td>0.005</td>
</tr>
<tr>
<td>HIV viral load &lt; 200 copies/mL</td>
<td>20 (18:25)</td>
<td>20 (18:25)</td>
<td>20 (18:25)</td>
<td>1.0</td>
</tr>
<tr>
<td>Number of mucosal sites involved at presentation</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>1.0</td>
</tr>
<tr>
<td>Hospitalized at symptom day 7</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>1.0</td>
</tr>
<tr>
<td>Development of severe mppox after symptom day 7</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>1.0</td>
</tr>
<tr>
<td>Development of severe mppox after symptom day 7</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>10 (5-18)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Table:** Mppox Virus Model of Sexual Transmission in Cynomolgus Macaque

Cécile Herate, Audrey Ferrier Rembert, Francis Relouzat, Hélène Letcher, Quentin Pascal, Benoît Delaché, Olivier Ferraris, Roger Le Grand, Jean-Nicolas Tournier

**University Paris-Saclay – Site de Fontenay-aux-Roses, Fontenay-aux-Roses, France, Institut de Recherche Biomédicale des Armées, Bretigny-sur-Orge, France, Institut de Recherche Biomédicale des Armées, Bretigny-sur-Orge, France, Vaccine Research Institute, Fontenay-aux-Roses, France**

**Background:** The Monkeypox virus (MPXV) responsible for 2022 outbreak belongs to clade IIB which derives from the less virulent clade and whose precise evidence and model on the potential sexual transmission of MPX remains limited and do not fully reflect the pathophysiology of the virus infection. In particular, there are no precise evidence and model on the potential sexual transmission of MPX. Today, the animal models of MPX and more particularly the non-human primate models (NHP) available remains limited; and do not reflect the pathophysiology of the virus infection. In particular, there are no precise evidence and model on the potential sexual transmission of MPX.

**Methods:** We mimicked virus transmission during sexual activities by challenging Cynomolgus macaques by intrarectal (ID) or intrarectal (IR) route...
alone or in combination with a MPXV strain isolated on a French patient in 2022. Infection of NHPs was documented clinically (clinical score and photographs) and monitored by blood count. Virus dissemination was tested by qRT-PCR and viral isolation, and the humoral immune response was assessed by specific IgG quantification and seroneutralisation. The virus was tested in various compartments, including blood, seminal fluid, rectal secretions and saliva. In a second time, we focused on the IR transmission and have mapped the virus-infected organs and performed large histology analysis.

Results: We observed a moderate disease whatever the route of infection with clinical symptoms similar to human including fever episodes, lymphadenopathy and skin lesions. Interestingly, the kinetics of clinical symptoms appearance is also very similar to human. The infection was characterized by marked lymphopenia, combined with monocyteosis and a drop in Hemoglobin as reported in patients. Virus was detected in skin lesions, blood, rectal mucosa and semen. Sexual fluids contain infectious virus during 2 weeks after IR transmission. We also observed that IR challenge provoked a systemic infection with virus dissemination in organs including digestive tract and genital tract but a very limited seroconversion. Histological analysis also revealed remarkable inflammation and infiltration in the infected organs such as rectum and genitocrural tract.

Conclusion: We have set up a non-lethal model of MPox in NHPs by intrarectal route and validated that Mpxo can be considered as a sexually transmitted disease disseminated by sexual fluids. We established a pathophysiological model of Mpxo with symptoms similar to human that will allow to assess vaccines and therapeutics against this emerging disease.

422 Tecovirimat Plasma Concentrations, Mpxo Resistance Mutations Selection, and Clinical Outcomes
Stephanie Marot, Minh P. Le, Nicolas Visinoni, Claire Perillaud-Dubois, Roxandra-Dana Calin, Vincent Berot, Valentin Ledurq, Cécile Poudroux, Olivier Ferraris, Laurence Murand-Joubert, Vincent Calvez, Gilles Pialoux, Gilles Peytavin, Anne-Geneviève Mareclini, Valérie Fourcher
Hôpitaux Universitaires Pitié Salpêtrière, Paris, France; Hôpital Bichat-Claude-Bernard, Paris, France; Saint-Antoine Hospital, Paris, France; Renon Hospital, Paris, France; Institut de Recherche Biomédicale des Armées, Bertigny-sur-Oise, France; Assistance Publique–Hôpital de Paris, Paris, France
Background: During the recent Mpxo outbreak, Tecovirimat was used to treat patients with severe disease. There are few studies characterizing Tecovirimat resistance mutations and none describing concomitant drug plasma concentrations.

Methods: Two HIV-1 infected patients hospitalized for severe and persistent Mpxo clinical manifestations were studied. Sequential sampling of several anatomic compartments were screened for Mpxo by PCR. Positive samples were sequenced using whole genome sequencing. Plasma concentrations of Tecovirimat were determined using LC-MS/MS (lower limit of quantification - LLOQ: 1 mg/L).

Results: Patient 1 with a low CD4+ cells count (198 CD4+/mm3) despite an undetectable viral load, presented multiple Mpxo skin lesions, evolving with fever and associated with extensive bilateral pulmonary, hepatic Mpxo lesions and persistent Mpxo viremia, motivating a Tecovirimat treatment. Mpxo clinical evolution was very slow but favorable after 90 days of Tecovirimat (600mg BID). Whole genome sequencing showed no resistance associated mutations (RAMs) and Tecovirimat plasma concentrations were in the expected range (177 and 1635 mg/L) during all the follow-up. Patient 2 presenting an uncontrolled HIV-1 replication (30,000 copies/mL) and a deep immunodeficiency (43 CD4+/mm3) during all the follow-up.

Conclusion: Persistent replication of Mpxo under Tecovirimat pressure in an immunosuppressed patient harboring adequate plasma drug concentrations was not associated with RAM selection. However, in case of suboptimal Tecovirimat plasma concentrations, we observed in another patient a rapid selection of RAMs associated to a clinical and virological rebound. These low plasma concentrations could be due to adherence issues, malabsorption or high BMI. This study highlights the interest of the Therapeutic Drug Monitoring of Tecovirimat in special populations and raises the discussion of dose adjustment.

423 Predictors of Treatment With Tecovirimat and Hospitalization Among People With Mpxo and HIV
Michalina Montano, Adrienne E. Shapiro, Rob Frederiksens, Heidi M. Crane, Nina Kim, Richard D. Moore, George A. Yendewa, Laura Bamford, Greer Burkholder, Katerina Christopoulos, Kenneth H. Mayer, Sonia Napramik, April Pettiti, Mari Kitahata, Rachel A. Bender Ignacio
1Fred Hutchinson Cancer Center, Seattle, WA, USA, 2University of Washington, Seattle, WA, USA, 3The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4Case Western Reserve University, Cleveland, OH, USA, 5University of California San Diego, La Jolla, CA, USA, 6University of Alabama at Birmingham, Birmingham, AL, USA, 7University of California San Francisco, San Francisco, CA, USA, 8Fenway Health, Boston, MA, USA, 9University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 10Vanderbilt University, Nashville, TN, USA
Background: Thousands of mpxo cases have been reported in the US since 2022, disproportionately affecting people with HIV (PWH), but epidemiologic data on mpxo among PWH are limited. We examined demographic and clinical characteristics of PWH with mpxo across the US in the Center for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort.

Methods: We studied all PWH in care at 9 CNICS sites and identified mpxo cases based on: a positive mpxo PCR value, a documented mpxo diagnosis, or tecovirimat prescription without accompanying negative mpxo PCR result between June-December 2022. We examined the most proximal CD4 count and HIV viral load (VL), as well as antiretroviral therapy (ART) status prior to mpxo diagnosis. We used generalized linear models with Poisson distribution and robust variance to estimate associations of demographic and clinical characteristics with tecovirimat prescription and hospitalization within 2 weeks of mpxo diagnosis, adjusting for age and site.

Results: We identified 381 PWH who had mpxo, all assigned male at birth, with median age of 39 years; 30.7% identified as non-Hispanic White, 22.3% as non-Hispanic Black, and 29.9% as Hispanic. A majority of mpxo cases were prescribed ART (95%, Table), had undetectable HIV VL (77%), and a CD4 count ≥500 (55%) at mpxo diagnosis. Most mpxo diagnoses (77%) were confirmed by PCR and 13% of cases had received ≥1 doses of JYNNEOS vaccine prior to mpxo diagnosis. PWH with mpxo who had CD4 count <200 were twice as likely to receive Tecovirimat compared to those with CD4≥500 (adjusted prevalence ratio [aPR]: 2.0, 95% confidence interval [CI]: 1.5-2.8). Race/ethnicity, detectable HIV VL, and ART status were not associated with Tecovirimat receipt. Cases with lower CD4 count were more likely to be hospitalized within 2 weeks of mpxo diagnosis compared to those with CD4≥500 (CD4≥200: aPR: 2.1, 95%CI: 0.7-6.6; CD4 200-350: aPR: 2.6, 95% CI: 1.2-5.8); those not on ART were nearly 4 times more likely to be hospitalized (aPR: 3.9, 95% CI: 1.7-8.9) compared to those prescribed ART, and those with detectable HIV VL were twice as likely to be hospitalized (aPR: 2.1, 95% CI: 1.1-4.3) as those with viral suppression.

Conclusion: Among PWH at HIV care centers across the US, we observed notable associations between CD4 count <350, detectable VL, lack of ART and hospitalization following mpxo diagnosis, suggesting that immunologic risk may extend beyond those with CD4 <200, the threshold at which many PWH are prescribed tecovirimat.

Clinical characteristics of PWH with mpxo across 9 sites of the CFAR Network of Integrated Clinical Systems diagnosed from June – December 2022.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No (%)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 count &lt; 200, cells/mm³</td>
<td>25</td>
<td>6.6</td>
</tr>
<tr>
<td>CD4 count 200-349 cells/mm³</td>
<td>47</td>
<td>12.3</td>
</tr>
<tr>
<td>Detectable HIV viral load</td>
<td>75</td>
<td>19.7</td>
</tr>
<tr>
<td>On ART</td>
<td>353</td>
<td>94.6</td>
</tr>
<tr>
<td>Tecovirimat prescribed</td>
<td>165</td>
<td>43.3</td>
</tr>
<tr>
<td>Received 1 JYNNEOS dose prior to mpxo diagnosis</td>
<td>34</td>
<td>8.9</td>
</tr>
<tr>
<td>Received 2 JYNNEOS doses prior to mpxo diagnosis</td>
<td>17</td>
<td>4.5</td>
</tr>
<tr>
<td>Hospitalized within 2 weeks following mpxo diagnosis</td>
<td>38</td>
<td>9.2</td>
</tr>
</tbody>
</table>

1CNICS sites contributing to this cohort were in Baltimore, Birmingham, Boston, Chapel Hill, Cleveland, Nashville, San Francisco, San Diego, and Seattle.
**Predictors of Mpx Duration and Severity in the Italian Multicenter Mpx Icona Cohort**

Valentina Mazzotta, 1 Silvia Nozza, 1 Simone Lanini, 1 Davide Moschese, 1 Alessandro Tavelli, 1 Roberto Rossotti, 1 Fusco Francesco Maria, 1 Lorenzo Biasioli, 1 Giulia Matsusali, 1 Angelo Roberto Raccagni, 1 Davide Mileto, 1 Antonella D’Amirino Monforte, 1 Antonella Castagna, 1 Andrea Antinori, 1 for the Mpx Icona Study Group

1IRCCS Lazzaro Spallanzani, Rome, Italy, 1San Raffaele Scientific Institute, Milan, Italy, 1Luigi Sacco University Hospital, Milan, Italy, 1Tosco Foundation, Milan, Italy, 1ASTS Grande Ospedale Metropolitano Niguarda, Milan, Italy, 1ARN in Colli, Naples, Italy, 1Azienda Ospedaliera San Paolo, Milan, Italy, 1Lazzaro Spallanzani National Institute for Infectious Diseases, Rome, Italy, 1Fondazione Istituto Storico Italiano. Milan, Italy

**Background:** The Mpx 2022 outbreak was characterized by novel clinical presentation and new routes of transmission. We explored potential predictors of Mpx severity and duration and described Mpx virus (MPXV) persistence in relevant biological fluids after healing.

**Methods:** Italian multicenter study in the network of Icona cohort. Severe Mpx cases were defined as hospitalized or proctitis, pharyngitis, otitis, ocular lesion, or >20 skin lesions; recovery as the resolution of all mucocutaneous lesions. Mixed effect regression models with a random intercept for clinical center assessed predictors of Mpx duration (linear model) and severity (logistic model). A stepwise backward process (with p=0.200) was fitted for the selection of variables in both multivariable models, using HIV status as a priori predictor. Assessment of early MPXV viral load (VL) as a predictor of severity was done by Student T-Test and mixed effect logistic regression model including random intercept on clinical center.

**Results:** 541 pts: 99.2% male, median age 38 (IQR 33-44), 43.5% PLWH (see Fig1A). Mean Mpx duration was 23.1 days (95% CI 21.9-24.3), significantly longer in pts with lymphadenopathy (+2.47 days), sore throat (+3.12), proctitis (+4.78), diffuse rash (+3.42) and in PLWH with <350 CD4 (+12.51) (Fig1B). Caucasian race (OR 1.82), fever (OR 1.95; p<0.002), lymphadenopathy (OR 2.30), sore throat (OR 2.14), and peri-anal lesions (OR 2.91) at onset were significantly associated with severe Mpx (Fig1C). Quantitative determination of VL in the upper respiratory tract (URT) was available for 233 pts (136 mild and 97 severe). Mean Ct-value was 37.3 (33.9-40.8) and 34.6 (30.9-38.3) in mild and severe cases, respectively (P<0.005), and the probability of developing severe disease was inversely associated with Ct-value, dropping by 5% per Ct (OR 0.95; 0.91-0.98; p<0.005; Fig1D). We found no association between severity and VL in other fluids. Detectable MPXV was found in sperm (14/60 pts), urine (5/86), anorectal (10/72), and URT (24/160) up to 46 days after recovery with a minimum Ct value of 26.

**Conclusion:** In the event of proctitis, sore throat, lymphadenopathy, and disseminated skin lesions, Mpx may last longer, as well as in PLWH with a low CD4 count. Caucasian race and presentation with fever, sore throat, lymphadenopathy, and peri-anal lesions predicted disease severity directly associated with VL in URT. Still unclear if viral shedding after recovery in several anatomical sites could lead to persistent infectivity.

**Molecular Epidemiology of Human Mpx in Chicago**

Lacy M. Simons, 1 En-Ling Wu, 1 Marlon Bolon, 1 Timothy Blanke, 1 Maria Francesca Agnes, 1 Anjelo M. Evans, 1 Arghavan Alisaotamdehkhordi, 1 Chad Achenbach, 1 Valentina Stosser, 1 Kendall Kling, 1 Egon Ozer, 1 Judd F. Hultquist, 1 Ramon Lorenzo-Redondo

Northwestern University, Chicago, IL, USA

**Background:** Mpx (formerly known as monkeypox) is a syndrome of fever, rash, and lymphadenopathy caused by the Mpx virus (MPXV), a zoonotic double-stranded DNA orthopoxvirus. The virus was first isolated in monkeys from Singapore in 1959 and is now known to have several animal reservoirs. Since its emergence in humans in the 1970’s, MPXV has become endemic in central and western Africa. In 2022, an outbreak of Mpx consisting of over 88 thousand cases across 113 countries prompted the World Health Organization to declare a public health emergency of international concern. High-risk populations for transmission were prioritized for vaccination with the JYNNEOS MPXV/smallpox vaccine. Global case counts peaked in early September of 2022 before tapering off in January 2023. In April 2023, a small resurgence of cases was noted in Chicago, including several vaccinated individuals, raising concerns of immune escape.

**Methods:** In this study, we investigated the genetic variation and evolution of the virus throughout the 2022-2023 outbreaks in Chicago. Between July 1, 2022 and September 1, 2023 residual diagnostic specimens were collected from Northwestern Memorial Hospital and affiliated clinics for DNA extraction and whole genome sequencing. In total, 139 specimens from 98 patients were collected, and 101 specimens from 77 patients were of sufficient viral load for whole genome sequencing.

**Results:** We observed a rapid increase in viral population size and diversity peaking in August 2022, comprised of eleven lineages, indicative of a high degree of viral circulation. On the contrary, all of the 2023 cases belonged to the same lineage, B.1.20, defined by three synonymous mutations. These most likely arose from a single transmission cluster, which was supported by traditional epidemiological follow up.

**Conclusion:** The circumstances underlying the unprecedented, international outbreak of Mpx in 2022 remain to be fully elucidated. The reemergence of MPXV in 2023 suggests continued circulation in the human population, which is supported by the virus’ continued evolution. Ongoing and robust epidemiological and genomic surveillance is required to determine if MPXV has become endemic in populations outside of West Africa. Continued monitoring of the genetic variation of the virus will allow for development of new antivirals and vaccinations with increased efficacy.

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1281 STIs were diagnosed from January to December 2022, of whom 654 in PLWH, 627 in PrEP-users. STIs peaked as follows: Chlamydia infection in October (49 cases), followed by January and July (42 and 45 cases); gonorrhea in June (38), November (37) and January (36), syphilis in August (35 cases), February and March (34 each); Mpx in June and July (49 cases) (Figure 1).

**Conclusion:** We found that classical STIs had a fluctuating, still constant presence throughout the year, while Mpx showed a decline even if, in the same period, the mentioned events continued to be held. These findings suggest the
unlikeliness of a behavioural change in influencing such a decline, putting in a new light the role of vaccination in the MSM community.

**427 Mpx Vaccine Uptake Among MSM During the 2022 Epidemic: A Single-Center Retrospective Study**

Yanis Merad, Matthieu Godinot, Dulce Alfaiate, Agathe Becker, Florence Ader, Anne Conrad, Laurent Cotte
Hôpitaux Civils de Lyon, Lyon, France

**Background:** During the 2022-2023 Mpx outbreak, 5010 cases were reported in France. Among them, an estimated 85% occurred in men who have sex with men (MSM). Smallpox vaccination was offered to at-risk populations in France, including MSM, starting July 2022. We sought to assess the vaccine uptake rate in MSM and the factors associated with vaccination uptake in different MSM risk-groups: people living with HIV (PWH), people who use pre-exposure HIV prophylaxis (PrEP), and other individuals. PrEP non-PrEP users.

**Methods:** A retrospective observational study was conducted in Lyon, France, enrolling MSM presenting at the sexual health clinics of the University Hospital between July 1st, 2022 and February 28th, 2023. Data regarding HIV infection, PrEP use, Mpx infection and Mpx vaccination (first dose received) were collected from electronic medical records. All patients gave informed consent regarding vaccination and the use of their data for analysis.

**Results:** A total of 9256 MSM were enrolled, including 1946 PWH, 2528 PrEP users and 4782 NPU. The median age of each group was respectively 51, 34 and 29 years. The vaccination rate of all participants at the end of follow-up was 49.6%. Compared to NPU, it was significantly higher for PrEP users (72.2%) with a relative risk estimate of 1.62 (95%CI 1.56-1.69), and significantly lower for PWH (32.7%) with a relative risk estimate of 0.73 (95%CI 0.68-0.79). Of note, half of PrEP users were vaccinated by day 66 of the vaccination campaign. In multivariate cumulative risk analysis, HIV infection/PrEP use, age and chemsex were all independently correlated with vaccination uptake.

**Conclusion:** Our study demonstrates Mpx vaccination uptake in MSM during the 2022 outbreak in France was high, especially in PrEP users. This could be explained either by the closer follow-up of this group, or their proactive engagement in STI prevention strategies. In contrast, PWH, although regularly followed-up, were less receptive to the vaccination campaign. It is likely that sexual behavior of PWH, hence exposure risk, are noticeably different. Although vaccination is probably not the only explanation, these successful results mirror the sharp decrease of Mpx cases reported after August 2022 in France.

428 Pharmacokinetics of Tecovirimat in Persons With Mpx: Results From ACTG 5418

1University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 2University of California Los Angeles, Los Angeles, CA, USA, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 4Massachusetts General Hospital, Boston, MA, USA, 5The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 6DHL Corporation, Atlanta, GA, USA, 7National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 8Stony Brook University, Stony Brook, NY, USA, 9University of California San Diego, La Jolla, CA, USA, 10Weill Cornell Medicine, New York, NY, USA, 11Columbia University Medical Center, New York, NY, USA

**Background:** Tecovirimat is an FDA-approved oral antiviral medication for human smallpox and is being used widely for Mpx treatment. The pharmacokinetics (PK) of tecovirimat has been assessed in animal models and healthy human volunteers, but data in people with Mpx are limited.

**Methods:** The Study of Tecovirimat for Mpx (STOMP/ACTG 5418, NCT05534984) is an ongoing international randomized (2:1) clinical trial of the efficacy of tecovirimat for Mpx. STOMP includes an open-label arm of participants with severe disease, immunosuppression, pregnancy and those aged <18 years, which is the focus of this report. Intensive PK assessments were conducted in a subset of participants on study day 8 (steady-state) following an observed dose of tecovirimat with a meal. All received 600 mg twice daily. Blood samples were collected pre-dose (time 0), 1, 2, 3, 4, 6, 8, and 10 hours post-dose. Tecovirimat concentrations were quantified by LC-MS/MS. PK were analyzed via noncompartmental analysis with predicted AUC0-12h and C12h determined from the terminal phase of the post-dose curve. AUC0-12h and C12h were compared to predicted exposures in fed healthy adults receiving the same doses (data from the U.S. FDA) and mean Cmin values associated with survival and clinical/virologic response in non-human primates (NHP) (minimum and maximum effective doses of 3 and 10 mg/kg/day, respectively).

**Results:** PK data were generated in 13 participants (11 non-pregnant adults; 1 adolescent; 1 pregnant female adult). Of 12 non-pregnant participants, 11 were male, 5 were Black; 7 were persons with HIV of whom 4 had HIV-1 RNA <200 copies/mL, 2 were not on ART, and 2/6 had CD4 counts <200 cells/mm³; median (range) age and weight were 35 (<18-51) years and 77 (57-103) kg, respectively. Comparable PK was observed between non-pregnant participants and the pregnant participant and by HIV status (Table). Median tecovirimat AUC0-12h and C12h in non-pregnant adults were 38% and 72% lower than 50th percentile exposures in healthy adults. Despite lower tecovirimat exposures, C12h across all participants exceeded mean effective Cmin values in NHPs receiving 3 and 10 mg/kg/day.

**Conclusion:** Persons with Mpx had lower tecovirimat exposures vs. healthy adults, although concentrations remained above the minimally effective Cmin in NHPs. Future work will explore factors associated with tecovirimat PK and assess relationships with clinical and virologic outcomes to evaluate the clinical relevance of these PK differences.
429 Evaluation of a Protocol for Managing Cyclosporine and Nirmatrelvir/ Ritonavir Drug-Drug Interaction

Pierre Giguere1, Kyla Agtarap1, Marie-Josée Deschênes1, Lacey DeVeere3, Jessica McDougall1, Stephanie Hour1, Swapnil Hiremath1

1The Ottawa Hospital, Ottawa, Canada, 2Hospital Montfort, Ottawa, Canada, 3University of Ottawa, Ottawa, Canada

Background: Morbidity and mortality of COVID-19 is higher in immunocompromised people including kidney transplant recipients (KTR). Nirmatrelvir/ritonavir (NRMr) interacts with many medications, in particular calcineurin inhibitors (CNI). Limited evidence exists for reintroducing CNIs post-NRMr. We created a protocol to manage the NRMr-cyclosporine (CsA) interaction. This study aims to describe the application of the protocol and deviations, evaluate its impact on cyclosporine levels, and analyze safety outcomes.

Methods: This is a retrospective study between May 2022 and Nov 2023. As per protocol, CsA dose was reduced by 80% at NRMr start and up to 2 days post-NRMr. CsA levels were drawn 2 days post-NRMr. If the 1st level was sub/therapeutic, CsA was resumed at 100% and level repeated 1 week later. If supratherapeutic, the dose reduction was maintained for another 2 days, then reintiated at 100% pre-NRMr dose. Acute kidney injury (AKI), hospitalization and death were collected through 30-days post-NRMr therapy.

Results: Forty-two KTR were identified, of which 35 started NRMr and met eligibility criteria and were analyzed. Nine KTR did not follow the protocol. Though CsA levels outside therapeutic range were common (9% and 31% vs 9%), 2 patients with sub and supratherapeutic CsA levels were resolved with increased fluid intake. There were 4 hospitalizations, but none related to COVID-19 treatment (unstable atrial flutter, gastroenteritis) and one that resolved with increased fluid intake. Of the 16 patients requiring additional monitoring, levels returned to baseline values within 30 days. Two KTR experienced AKI for unrelated reasons (20% vs 31% highest). CsA level was higher by 23 μg/L (P=0.51). There was a greater proportion of levels outside therapeutic range. At the second FU, there was a correction of sub and supratherapeutic levels compared to baseline (20% vs 31% vs 9%).

Nonadherence to the protocol was observed in 5/18 levels. Data presented as median (interquartile range). CNIs: pre-NRMr inclusions in patients under treatment conditions. *Ratio of median values *Reported is 5 with 4 without NRMr

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<th>Parameter</th>
<th>Pregnant (n=18)</th>
<th>Non-Pregnant (n=18)</th>
<th>Total (n=36)</th>
<th>Healthy Adults*</th>
<th>Non-Pregnant vs. Healthy Adults*</th>
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<td>CsA (mg/L)</td>
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<td>305 (13.1)</td>
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<td>CsA (mg/L)</td>
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<td>0.24</td>
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Conclusion: Adherence to the protocol is crucial. Though CsA levels outside therapeutic range were common immediately post-NRMr treatment, levels were restored rapidly. No clinically relevant safety events related to NRMr treatment occurred within the 30-days. Adherence to the protocol is crucial.

430 Disparities in Mpxo Vaccination Among Cis Men and Trans Persons With HIV in Los Angeles County

Colleen Leonard1, Erin Nguyen1, Kathleen Poortinga2, Sherry Yin1, Andrea A. Kim1, Olivia Moir1, Natalie Frey1, Abraar Karan2, Sonali Kulkarni1, Rebecca Cohen1, Kiva Sey1, Shobita Rajagopalan3, Mario J. Pérez1

1Los Angeles County Department of Public Health, Los Angeles, CA, USA, 2Stanford University, Stanford, CA, USA

Background: The CDC recommends the 2-dose JYNNEOS vaccine for protection against mpxo for people with HIV (PWH) due to a high proportion of US mpxo cases occurring in PWH and an increased risk of severe mpxo disease among those with uncontrolled HIV infection. In Los Angeles County (LAC), 45% of mpxo cases have occurred in PWH, primarily among cis-men and transgender persons. We aim to identify demographic and health-related predictors of JYNNEOS vaccination among cis-men and transgender PWH in LAC.

Methods: Cis-men and transgender adults aged ≥ 18 years with diagnosed HIV in LAC were included. Unvaccinated PWH with a prior mpxo diagnosis were excluded. Potential predictors of JYNNEOS vaccination included: demographics, viral suppression, CD4 count, HIV transmission mode, receipt of HIV care (past 12 months), retention in HIV care*, recent STI diagnosis, recent COVID vaccination (proxy for individual vaccine confidence). Fully nonproportional odds models were used to measure the associations between predictor variables and the outcome (2, 1 or 0 doses of JYNNEOS).

Results: Among 37,613 cis-men or transgender PWH, 8,757 (23.3%) were fully vaccinated, 3,473 (9.2%) had received 1 dose, and 25,383 (67.5%) were unvaccinated. Disparities by age group, race/ethnicity, and healthy places index (HPI) were observed. Full vaccination was lowest among PWH who were aged 18-29 (14.5% vs 26.2% aged 40-49), Black (17% vs 30.3% Asian and 28.8% White), or living in zip codes in the lowest quartile of HPI (20% vs 31% highest). Prior COVID vaccination (adjusted odds ratio [aOR] 2.9, confidence interval [CI] 2.7-3.2), receipt of HIV care in the past 12 months (aOR 2.3, CI 1.9-2.7), recent STI diagnosis (aOR 1.6, CI 1.5-1.7), and living in the highest HPI quartile (aOR 1.5, CI 1.4-1.6) were significant predictors of JYNNEOS vaccination (Figure). Black (aOR = 0.67, CI: 0.62-0.73) and Latinx (aOR = 0.73, CI: 0.68-0.78) PWH were less likely to be fully vaccinated against mpxo compared to White PWH.

Conclusion: Patients engaged in HIV care or who had a recent STI diagnosis were significantly more likely to receive the JYNNEOS vaccine; this may be attributable to vaccine receipt during an HIV/STI visit or underlying individual healthcare seeking behaviors. These findings also highlight the importance of tailoring vaccine outreach efforts to improve vaccine confidence and access, especially for younger individuals, underserved neighborhoods, and Black and Latinx communities.

431 A Novel Non-Invasive Approach to Sample Female Genital Tract Immune Cell Populations

M Quinn Peters1, Eva Domenico-Yáñez1, Melanie Gazper1, Smrittee Dabee1, Blair Armstead1, Christopher Whidbey2, Heather Jagan3, Martin Pric1, Whitney E Harrington1

1Seattle Children’s Research Institute, Seattle, WA, USA, 2Fred Hutchinson Cancer Center, Seattle, WA, USA, 3University of Washington, Seattle, WA, USA

Background: An increased understanding of immune cell populations in the female genital tract (FGT) is essential for efforts to decrease risk of HIV and other sexually transmitted infections and to develop vaccines that induce mucosal responses. To-date, FGT immune cell collection has utilized techniques that require a healthcare provider and a speculum exam, creating a barrier to...
participation and limiting longitudinal studies. To overcome these challenges, we developed a novel method to collect leukocytes from cervicovaginal fluid (CVF) utilizing Softdiscs. 

**Methods:** Non-menstruating persons with vaginas of reproductive age were asked to self-insert disposable Softdiscs and wear for up to 4 hours on three sequential days. After self-retrieval, CVF was removed from the disk via washing, followed by treatment with DTT to thin mucus, passage through a cell strainer, and cryopreservation. Cells were subsequently thawed, enriched for CD45+ cells using magnetic cell separation, and stained with a 28-color antibody panel focused on T cell phenotyping.

**Results:** Across 15 samples (5 participants x 3 days) we recovered high numbers of CD45+ cells (median: 6,233; range: 212-57,703), CD3+ T cells (median: 1,182; range: 12-11,554), and CD19+ B cells (median: 1,072, range: 8-4,690). Of CD3+ T cells, frequencies ranged from 37-87% for CD4+ T cells and 7-55% for CD8+ T cells. Within CD4+ there were both regulatory (Tregs) and conventional subsets and within CD8s both tissue resident (TRM) and migratory populations. To demonstrate reproducibility, we compared populations across days and found that while individuals the distribution was strongly conserved. For example, comparing the first and last day of sampling, the frequency of CD4s (R2=0.78, p=0.12) and CD8s (R2=0.90, p=0.04) of CD3s, Tregs of CD4s (R2=0.75, p=0.15), and TRM of CD8s (R2=0.93, p=0.02) were all correlated. Of note, while population distributions were strongly conserved within individuals, they were unique across individuals (Fig 1).

**Conclusion:** Collection of CVF from Softdiscs represents a novel, non-invasive approach to study FGT immune cell populations, including both tissue resident and migratory memory T cell subsets. This approach yielded high quality immune cells which could be cryopreserved and had reproducible population structure and phenotype across multiple days. This self-collected sampling will empower a more diverse population of individuals to participate in future studies of FGT immunity.

**432 Identification of Innate Lymphoid Cell Subsets in the Human Female Genital Tract That Respond to HIV**

Alexandra E. Werner, Laura Moreno de Lara, Francisco J. Carrillo-Salinas, Anna Borchers, Siddharth Parthasarathy, Marta Rodriguez-Garcia

**Background:** Women acquire HIV mainly through sexual contact. Identification of innate immune mechanisms that protect against infection in the female genital tract (FGT) will enable strategies to prevent HIV acquisition. Innate lymphoid cells (ILCs) are tissue-resident cells specialized in cytokine secretion that provide mucosal protection against infections in mice. However, human ILCs remain poorly characterized and their potential role in HIV prevention is unknown.

**Methods:** Human hysterectomies (n=35) were enzymatically digested to generate mixed-cell suspensions from endometrium, endocervix, and ectocervix. ILC phenotype and function was assessed with high-dimensional spectral flow cytometry for surface and intracellular molecules (22-parameters). To define ILC-mediated anti-HIV responses, genital mixed-cell suspensions were stimulated with HIV for 30 minutes in vitro, and cytokine content (IL-22 and IFNγ) and degranulation (CD107a) were analyzed by flow cytometry.

**Results:** Genital ILCs represented <5% of mononuclear immune cells. Subset distribution was compartmentalized, with ILC3s predominant in the endometrium (63% of ILCs), and ILC1s in the ectocervix (48%). In the absence of stimulation, ILC1s constitutively produced IFNγ (14%), while ILC3s produced IL-22 (46%), important for barrier maintenance. We identified two ILC3 subsets at steady state, CCR6+ and CCR6−, with the highest IL-22 content (63%; p=0.003), and highest degranulation (CD107a, 10.4%; p=0.002), respectively. Genital ILCs expressed HIV coreceptors (CCR5, CXCR4), but CD4 expression was restricted to ILC3s (p=0.001). In vitro HIV stimulation increased the percentage of CD107a+ ILCs (p=0.03), concomitant with a decrease in intracellular IFNγ (p=0.02), indicating IFNγ release and degranulation in response to HIV. For ILC3s, in vitro HIV stimulation induced a significant decrease in the percentage of CCR6+IL22+ cells, regardless of CD103 expression (p=0.03), while CD107a+ cells specifically increased in the CCR6+CD103− population (p=0.06), indicating a response to HIV through IL-22 release.

**Conclusion:** Our findings demonstrate that ILCs reside in the FGT, produce antiviral cytokines under steady-state, and ILC3s and CD103− ILC3s degranulate in response to HIV stimulation, indicating a major role in the maintenance of an antiviral environment and the ability to mount an antiviral response. Our investigation suggests ILCs could represent a novel mechanism for protection against HIV infection in the FGT.
Background: We previously demonstrated that Africans with variants in the Ig-like domain of CD101 are associated with an increased risk of heterosexually acquired HIV infection. Recognizing that CD101 has known immunoregulatory functions, we had shown that these CD101 Ig-like variants are associated with reduced regulatory T cell function and increased proinflammatory T cell responses in the circulation. We had not previously examined the effects of these variants on the genital mucosa - the portal of entry for sexually acquired HIV. Here, we explore associations between CD101 Ig-like variants and the level of soluble immune factors in genital secretions that may suggest a mechanism for how these variants impact HIV risk.

Methods: We enrolled women without HIV in HIV serodifferent heterosexual couples (with a male partner with HIV, N=44) or HIV seroconcordant negative couples (N=296) in Thika, Kenya consentin to collection of blood and genital samples. We used a custom Open Array (Thermo Fisher) to genotype ten candidate CD101 variants including six Ig-like variants (minor allele frequencies 0.007 – 0.11) and a Lumine assay (Eve Technologies) to measure 71 cytokines and chemokines in enrollment softcup and serum samples from 245 women. We used multivariable linear regression (controlling for frequency of condomless sex, for softcup) to compare the log cytokine and chemokine level in genital secretions or serum from women having one or more Ig-like variants (N=60)/245=24%) to women with none of the ten candidate CD101 variants (N=59).

Results: Between these two groups, median age 29 (18-54) years, women with one or more vs no CD101 Ig-like variants were associated with a significant reduction in levels of LIF (β=-0.41, p=0.001), G-CSF (β=-0.46, p=0.006), as well as IL-1α, PDGF-AB/BB, RANTES, Eotaxin-2, Eotaxin-3, IL-16, IL-33, and TPO (β=0.025 – 0.044) in genital softcup samples. In contrast, we found no significant association of CD101 Ig-like variants with any factors in serum.

Conclusion: Our exploratory findings suggest that CD101 Ig-like variants have immunoregulatory impact in the genital mucosa. Notably, RANTES, IL-16, and IL-14 have demonstrated HIV inhibitory activity. The reduction in these factors observed in genital secretions associated with CD101 Ig-like variants could undermine increased HIV infection risk. Additionally, G-CSF, PDGF-AB/BB, IL-33, and LIF facilitate tissue homeostasis and repair, so reduced levels could compromise mucosal tissue barrier and facilitate pathogen entry.

Synergistic Effects of HIV, HPV, and Polyomaviruses on Interferon Response in Male Anal Mucosa

Matteo Fraccella, Sara Passerini, Letizia Santinelli, Lorenzo Sorrentino, Luca Maddaloni, Ginevra Bugani, Eugenio Nelson Cavallari, Alessandra Pierangeli, Luca Pietropaolo, Carolina Scagnolari

Sapienza University of Rome, Rome, Italy

Background: Viral persistence is a crucial prerequisite for high-risk (HR) HPV-associated tumor growth, such as anal squamous cell carcinoma (SCC). Men living with HIV (MLWH) are more likely to be co-infected with HPV. Moreover, HIV may alter epithelial integrity, thereby favoring not only HPV but also Polyomaviruses (HPyVs) infections in basal cells, where they dysregulate cell division and local immunity. Indeed, HPyVs have garnered interest due to their carcinogenic potential and their possible presence in the anal mucosa, which could promote transmission through sexual intercourse. To explore the innate response of the anal mucosa in relation to HPV and HPyVs infections, we analyzed the expression of type I/II interferon (IFN-α/β) in anal cells from MLWH and non-MLWH.

Methods: Sixty-one anal canal brushing samples were collected from men attending a proctology clinic. Detection of HPV and HPyVs [i. Merkel cell PVy (MCPyV), ii. JC PVy, iii. BKPV] DNA was performed by PCR, and genotyping by sequencing. From purified cellular RNA, transcripts of genes encoding IFN-1α, β, IFN-3 (α1-3), and TLR9 were measured by quantitative RT-PCR assays and normalized to the housekeeping GUS gene.

Results: Thirty-five MLWH (mean age 47.7 years, SD 11.4), on long-term ART, and twenty-six controls (mean age 47.4 years, SD 15.3) were enrolled in this study. Out of the 61 men, 47 were HPV-positive, while 24 were HPyV-positive, with 87.5% specifically testing positive for MCPyV. HPV infections were associated with lower IFN-α mRNA levels compared to the HPV-negative group [Mann-Whitney (MW) test, p<0.05]. Notably, IFN-α levels decreased in HIV/HPyV co-infected patients [Jonckheere-Terpstra test (JT), p<0.006]. Furthermore, HPV/HPyV co-infections and the presence of low-grade Squamous Intraepithelial Lesion (LSIL) were associated with lower expression of TLR9 (JT, p<0.025). When comparing HPV/HPyV-positive and HPV/HPyV-negative men, IFN-α showed lower mRNA levels in the HPV/HPyV group (MW, p<0.007), while IFN-α was more produced in the HPV/HPyV group (MW, p<0.008).

Conclusion: The findings highlight the potential synergy between HPV and HPyV infections in compromising mucosal innate immunity and promoting viral co-infections, such as HPyVs, in the anal mucosa. Alterations in IFN and TLR-9 genes provide insights into complex mechanisms behind viral persistence, enhancing the understanding of innate immunity, viral infections, and HPV-related malignancies, with implications for future research and therapies.

Circulating Acyl-CoA-Binding Protein Abates T-Cell Function in People Living With HIV

Stephanie Isnard, Lena Roysdon, Tranollole Mabanga, Simeng Bu, Carolina A. Benini, Nicole F. Bernard, Julien van Grevenynghe, Jean-Guillaume Cordero, Jean-Pierre Routy

McGill University Health Centre Research Institute, Montreal, Canada, McGill University Health Centre, Montreal, Canada, NINS Centre Armand-Frappier Santé Biotechnologie, Laval, Canada, Centre de Recherche des Cordeliers, Paris, France

Background: Autophagy, a cytosolic-structure degradation pathway producing energy, allows efficient anti-HIV T-cell responses. Autophagy enables IL21 production in anti-HIV CD4 T-cells, which in turn stimulates lipophagy and enhances CD8 T-cell anti-HIV responses. Extracellular Acyl-CoA-Binding Protein (ACBP) inhibits autophagy, triacarboxylic acid (TCA) cycle and oxidative phosphorylation in mouse models. Herein, we assessed the levels of circulating ACBP and its influence on T-cell function in people living with HIV (PLWH) on antiretroviral therapy (ART).

Methods: Plasma ACBP and cytokines were quantified by ELISA in 50 PLWH on effective ART (mean duration 14.7 years) and 30 controls with similar age. Metabolomic analyses were performed on serum samples by GC-MS (10 participants with high and low ACBP). In vitro, recombinant ACBP (recACBP) was added at increasing concentration up to 100μg/mL on PBMCs from PLWH on ART and controls, T-cell responses were assessed by flow cytometry. Intracellular LC3B was visualized by microscopy.

Results: ACBP levels were higher in PLWH on ART compared to controls (median 127.5 vs 78.1 ng/mL, p=0.03), independently of age and sex. In ART-treated PLWH, plasma ACBP levels were neither associated with CD4 nor CD8 T-cell counts, however they correlated with levels of growth factors (EGF, G-CSF, GRO), pro-inflammatory cytokines (IFNα2, IFNγ, IL1β) and homeostatic factors (IL7 and IL15) (r=0.3, p<0.05 for all comparisons). Conversely, ACBP levels were inversely associated with plasma IL21 levels (r=-0.54, p<0.01). PLWH with high ACBP had higher serum levels of TCA intermediates glutamate (2-fold, p=0.02) and α-ketoglutarate (1.5-fold, p=0.09), respectively. We added recACBP to PBMCs stimulated with either anti-CD antibodies or HIV Gag, Nef and Env peptides for 16 h, and observed a decrease in IFNγ, IL2 and TNFα production while IL10 levels remained unaffected. RecACBP decreased intracellular levels of the autophagy marker LC3B without affecting cell viability.

Conclusion: Higher plasma ACBP levels in PLWH on ART were associated with inflammation, unif metabolism, and markers of T-cell dysfunction. Our findings indicate that circulating ACBP directly abates autophagy and anti-HIV T-cell functions, compelling the development of circulating ACBP inhibitors aiming at improving anti-HIV T-cell responses in PLWH, towards an HIV cure.

Immunological Non-Responders Have CD4+ Immunosenescence and Impaired Lymphocyte Cytokine Production


1Radboud University Medical Center, Nijmegen, Netherlands, 2OLVG, Amsterdam, Netherlands

Background: Immunological non-responders (INRs) are people living with HIV (PLWH) that fail to restore CD4+ T-cell counts despite suppressive antiretroviral...
Phenotype of Gamma-Delta T-Cells in Acute HIV-1 Infection Predict Neutralization Breadth

Gina L. Griffiths, Matthew Creegan, Kawthar Machmch Leggot, Margaret Constanzo, Isabella Swofford, Ningbo Jian, Leigh-Anne Eiler, Merlin L. Robb, Samantha M. Townsley, Shelly J. Krebs, Julie Ake, Dominic Faquin-Proulx, for the RV217 Study Group

Walter Reed Army Institute of Research, Silver Spring, MD, USA, University of Oxford, Oxford, United Kingdom, Kenya Medical Research Institute, Nairobi, Kenya, Imperial College London, London, United Kingdom, International AIDS Vaccine Initiative, New York, NY, Rwanda, Zambia HIV Research Group, Kigali, Rwanda, Uganda Virus Research Institute, Entebbe, Uganda, Lund University, Lund, Sweden, University College London, London, United Kingdom

Background: New HIV vaccine approaches are focused on eliciting broadly neutralizing antibodies (bNABs). Leveraging the U.S. Military HIV Research Program’s (MHRP) therapy naive RV217 cohort comprised of study participants monitored for HIV acquisition and progression, we have previously shown that B cell engagement and activation within the first 14–43 days of viremia are predictive of bNAB development. We therefore hypothesized that innate immune cells might provide signals to B cells during acute HIV-1 (AHI) infection resulting in neutralization breadth years later. This study characterized early innate immune responses in RV217 participants previously investigated for bNAB development.

Methods: Flow cytometry was performed on PBMC samples from 22 RV217 study participants previously studied for neutralization breadth development. Four timepoints per participant were utilized: pre-infection, days 14–25 post-infection (peak viral load), days 30–60 post-infection (viral set point), and >365 days post-infection (chronic infection). Data was analyzed utilizing global heat map analysis, univariate summary of marker analysis, longitudinal analysis of markers of interest, logistic regression analysis, cumulative incidence curves analysis, and area under receiver operating characteristic curve analysis.

Results: Flow cytometry analysis showed significant differences in gamma-delta (γδ) T cell surface marker expression in participants that developed neutralization breadth and those that did not. Levels of CD16 on γδ1 T cells were found to be significantly higher in broad neutralizers at set point viral load while levels of CD57 on γδ2 T cells were found to be significantly higher in broad neutralizers at both pre-infection and set point viral load. CD16 and CD57 are two markers associated with effector functions suggesting that more differentiated γδ T cells may contribute to the development of bNAs. Further analysis of these results revealed that these specific markers are significantly associated with the probability of bNAB development and are predictive of neutralization breadth accuracy at almost 80%. Our results identify CD16+ γδ1 T cells and CD57+ γδ2 T cells as potential key populations involved in the neutralization breadth development.

Conclusion: γδ T cells may play an important role in bNAB development during AHI and these results could inform HIV vaccine design.

NK Cells During Acute HIV Infection Are Associated With Slow Disease Progression in Subtype A


University of Oxford, Oxford, United Kingdom, Kenya Medical Research Institute, Nairobi, Kenya, Imperial College London, London, United Kingdom, International AIDS Vaccine Initiative, New York, NY, Rwanda, Zambia HIV Research Group, Kigali, Rwanda, Uganda Virus Research Institute, Entebbe, Uganda, Lund University, Lund, Sweden, University College London, London, United Kingdom

Background: Understanding immune responses linked with viral control during early HIV-1 infection is critical to develop new prophylactic and therapeutic strategies. HIV-1 subtypes impact disease outcomes, but their association with diverse immune responses during acute HIV-1 infection (AHI) is unclear. Natural killer (NK) cells contribute to HIV-1 control and may influence the pathogenesis of AHI. Using longitudinal samples collected during and after peak HIV-1 viral load (VL) in a prospective unique cohort of ART-naive individuals, we studied evolving NK cell responses in patients with different HIV-1 subtypes in relation to disease progression.

Methods: Participants with subtype A (n=28) and non-A subtype (n=17 subtype C and n=7 subtype D) were recruited from Kenya, Rwanda, Zambia, and Uganda between 2006-2011 under “IAVI Protocol C”. Samples were collected at two weeks (median=18 days), 1-month (median= 32 days), and 3-month (median=91 days) post-HIV-1 infection. Multiparameter flow cytometry was used for phenotypic characterisation. ADCC responses were determined against Raji cells. Soluble markers were evaluated using multiplexed assays.

Results: AHI induced expansion of NK cell subset with adaptive/memory features, as early as two weeks post-infection, increasing in magnitude after resolution of peak VL. This adaptive NK cell signature was delineated by lower expression of the signaling molecular FceRI and higher expression of the activating receptors NKGe2C and CD2. The kinetics of NK cell responses differed based on the infecting HIV-1 subtype, with patients with subtype A exhibiting a higher magnitude of adaptive NK cells than those with non-A subtype infection (p=0.036). A stronger innate immune response induced by subtype A, including IL-15 (p=0.021) and IL-12 (p=0.009), correlated with the enrichment of adaptive NK cells. This early increase in adaptive NK cells was associated with enhanced ADCC (p=0.002) and correlated with higher levels of CD8+ T-cell activation during AHI (r=0.621, p=0.015). Notably, higher frequencies of adaptive NK cells during the first month of infection were associated with future viral control in patients who maintained a persistently undetectable or low viral load (<10,000 copies/ml) up to six years post-infection.

Conclusion: These data provide insights into the beneficial role of adaptive NK cells during AHI, revealing previously unappreciated diversity of NK cell
responses to different HIV-1 subtypes, thereby informing strategies toward NK-cell-directed therapies.

**440 PWH on ART and Tyrosine Kinase Inhibitors Show High Cytotoxic Activity Against HIV-1–Infected Cells**

Claudia Sanz, Menendez1, Guoiam Casado Fernández1, Lorena Vigori1, Mario Manzanarez1, Elena Mateo1, Juan Ambrosioni1, Vicente Estrada1, Miguel Cervero Jiménez1, Valle Gómez1, Christoph Wyen1, Christian Hoffmann1, Montserrat Torres1, Verònica Bzza1, Vicente Planelles1, Mayte Coiras1

1 Instituto de Salud Carlos III, Majadahonda, Spain, 2Hospital Clinic of Barcelona, Barcelona, Spain, 3Hospital Universitario Clínico San Carlos, Madrid, Spain, 4Hospital Universitario Severo Ochoa, Madrid, Spain, 5Hospital Universitario de La Princesa, Madrid, Spain, 6Cologne University Hospital, Cologne, Germany, 7ICN Study Center, Hamburg, Germany, 8Institute of Health Carlos III, Madrid, Spain, 9University of Salt Lake City, UT, USA

**Background:** We evaluated if PWH with chronic myeloid leukemia (CML) on antiretrovirals (ART) and tyrosine kinase inhibitors (TKI) had cell populations with high antiviral capacity that may explain the reduced reservoir size observed in these individuals.

**Methods:** Blood samples were obtained from 6 PWH with CML on ART and 18 PWH on ART+TKI as controls. Cell immunophenotyping was performed by flow cytometry. Cytotoxic activity was determined using NK-specific target cells K562 and HIV-1-infected TZM-BL. Cytokine release was measured after stimulation with HIV-1 peptide pool. Statistical analysis was performed with Mann-Whitney or unpaired t-test.

**Results:** 1) Most participants were male (100% PWH on ART+TKI and 73% in PWH on ART). Cell immunophenotyping was performed by flow cytometry. Cytotoxic activity was determined using NK-specific target cells K562 and HIV-1-infected TZM-BL. Cytokine release was measured after stimulation with HIV-1 peptide pool. Statistical analysis was performed with Mann-Whitney or unpaired t-test.

**Conclusion:** Our findings indicate that HIV-associated NK cell irregularities persist in PWH despite long-term, effective ART. This underscores the need to better understand the causative mechanisms contributing to these immune irregularities. The study also provides a rationale for exploiting in HIV remission trials the selectively expanded antibody responsive Fcγ−NK cells that are maintained in PWH during ART.

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**441 A Skewed NK Cell Repertoire Persists in People With HIV-1 Despite Long-Term ART**

Renée R. Andersen, Allison E. DePuy, Rhi Bronson, Arelene C. Bullotta, Evgenia Aga, Ronald J. Bosch, B. Brad Jones, Joseph J. Erond, John W. Mellors, Rajesh T. Gandhi, Deborah K. McNeffon, Bernard Macintyre, Charles R. Rinaldo, Robbie B. Maelaid1, University of Pittsburgh, Pennsylvania, PA, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3Wellcome Cornell Medicine, New York, NY, USA, 4University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 5Massachusetts General Hospital, Boston, MA, USA

**Background:** HIV-1 infection can greatly impact the NK cell phenotypic and functional repertoire. This is highlighted by the expansion of a rare population of FcγR− NK cells exhibiting adaptive immune characteristics in people with HIV-1 (PWH). While current antiretroviral therapy (ART) effectively controls HIV-1 viremia and disease progression, its impact on HIV-associated NK cell abnormalities remains unclear.

**Methods:** We performed a 4-year longitudinal analysis characterizing conventional and memory-like NK cells from PWH (n = 60) participating in the AIDS Clinical Trials Group (ACTG) A5321 study, all of whom initiated ART in ACTG trials during chronic infection and had well-documented consistent HIV-1 suppression. Peripheral blood mononuclear cells collected at 4 weeks, 1 year, and 4 years post-initiation of ART were characterized. The phenotypic and functional profiles of NK cells, as well as frequencies of FcγR− NK cells, were determined by flow cytometry analysis at baseline and following a 24-h exposure to rhIL-18 (500 ng/ml) and rh-12p70 (50 ng/ml). Plasma CMV/EBV IgG antibody titers were measured by ELISA, and CMV/EBV viremia was determined by real-time qPCR. The BD Rhapsody™ Express System was used for single-cell multiomic analysis.

**Results:** Throughout the first 4 years of ART, a skewed repertoire of highly specialized antibody responsive but rh-IL-18–IL-12 unresponsive FcγR− memory-like NK cells persisted. This was accompanied by an increase in both CD57 and KLRG1 expression and a decrease in Nkp46 within the total NK cell population, indicative of a progressive differentiation toward senescence. These characteristics were linked to increasing antibody titers to CMV, with CMV viremia detected in 35% and 29% of PWH at years 1 and 4 of ART. Interestingly, 40% of PWH displayed an atypical NK cell subset distribution based on differential expression patterns of CD56 and CD16. Single-cell multiomic trajectory analysis (Figure) revealed that these abnormal subsets likely represent intermediate stages of NK-piosies, a phenomenon partially corrected between 1 to 4 years of ART.

**Conclusion:** Our findings indicate that HIV-associated NK cell irregularities persist in PWH despite long-term, effective ART. This underscores the need to better understand the causative mechanisms contributing to these immune irregularities. The study also provides a rationale for exploiting in HIV remission trials the selectively expanded antibody responsive Fcγ− NK cells that are maintained in PWH during ART.

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**442 Trained Immunity in Monocytes Is Associated With Persistent Elite Controller Status**

Jessica dos Santos, Suzanne Rujijert, Albert L. Groenendijk, Nadia Vadaq, Rainer Knoll, Robin T. Horst, Marc Blaauw, Louise E. van Eekeren, Willem A. Vos, Victoria Rios-Vazquez, Vasiliki Matzerak, Jan van Lunzen, Leo Joosten, Mihai Netea, Andre J. van der Veen

1 Radboud University Medical Center, Nijmegen, Netherlands, 2Erasmus University Medical Center, Rotterdam, Netherlands, 3University of Bonn, Bonn, Germany

**Background:** The protective role of trained innate immunity in various infections is well known, but its impact on HIV control has not been investigated. We hypothesized that trained monocytes, as induced by β-glucan, are crucial in orchestrating a beneficial antiviral immune response associated with long-term spontaneous HIV control.

**Methods:** 1895 people living with HIV, among which 114 HIV controllers (HIC) classified as Elite controller (EC), viremic controllers (VC) and transient controllers (TC), were included (2000HIV study - NCT03984835). First degree family members (FAM) of HIV controllers and non-HIV controllers (non-HIC) were part of the 2000HIV-trained study (NCT04968717). We analyzed multi-omics data (gene expression, DNA methylation, RNAseq) and functional profiles of NK cells, as well as frequencies of Fcγ− NK cells.

**Results:** Among 114 HIV controllers (HIC), 93 were EC, 20 were VC and 11 were TC. Their FAM were part of the 2000HIV-trained study (NCT04968717). We analyzed multi-omics data (gene expression, DNA methylation, RNAseq) and functional profiles of NK cells, as well as frequencies of Fcγ− NK cells.

**Conclusion:** Our findings indicate that HIV-associated NK cell irregularities persist in PWH despite long-term, effective ART. This underscores the need to better understand the causative mechanisms contributing to these immune irregularities. The study also provides a rationale for exploiting in HIV remission trials the selectively expanded antibody responsive Fcγ− NK cells that are maintained in PWH during ART.
Interestingly, HIC and their relatives displayed higher circulating concentrations of β-glucan, suggesting a trained phenotype in their monocytes. scRNA-seq demonstrated that monocytes of HIC displayed upregulation of type I IFN and antigen processing and presentation genes, as well as downregulation of genes associated with chronic inflammation and regulation of DNA transcription. Moreover, changes in gene expression levels and cytokine production of HIC, more prominently in EC, were sustained by changes in chromatin accessibility in innate immune genes, among which NF-κB and type I IFN were reported.

**Conclusion:** Monocyte responsiveness is increased in HIV controllers and their relatives, which is associated with trained immunity and related epigenetic regulation. These traits are stronger in EC compared to transient controllers, suggesting the importance of a trained program in monocytes in maintaining HIV control.

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**443**  
**Effective Elimination of HIV-1 Infected CD4+ T-Cells by NKG2C+ “Memory-Like” NK Mediated by Trail**

**Idelfonso Sánchez**, Marta Calvet Mirabent, Olga Popova, Ignacio de los Santos, Lucio J. García-Fraile, Ilya Tsukalov, Arantxazu Alfranca, María Buzón, María A. Muñoz-Fernández, Francisco Sánchez- Madrid, Enrique Martín-Gayo

1Hospital Universitario de La Princesa, Madrid, Spain, 2Hospital Universitario de la Vall d’Hebron, Barcelona, Spain, 3Hospital General Universitario Gregorio Marañón, Madrid, Spain, 4Universidad Autónoma de Madrid, Madrid, Spain

**Background:** “Memory-like” NKG2C+ Natural Killer (NK) cells from people with HIV-1 (PLWH) induced in the presence of dendritic cells (DC) are associated with a more effective elimination of autologous latently HIV-1 infected CD4+ T cells. TRAIL is a TNF family receptor that can induce cytotoxic function. In this study, we evaluated the expression of this receptor and its potential role in function of memory-like NK cells eliminating HIV-1 infected CD4+ T cells.

**Methods:** NK cells were isolated from the blood of n=19 PLWH on ART and activated in the presence of autologous monocyte-derived DC pre-treated with nanoparticles loaded poly I:C (Nano-PIC DC). Expression of TRAIL in different memory and effector NK cell subsets defined by NKG2C vs CD57 expression was tested and associated with functional ability to kill HIV-1 infected cells.

**Results:** CS6dim and CS6- CD16+ NK cells from PLWH exposed to nano-PIC DC displayed increased levels of TRAIL compared to cells stimulated with control nano DC (p=0.0039). Induction of TRAIL expression was higher in CS6dim and CS6- CD16+ NK cells from PLWH characterized by effective elimination of infected CD4+ T cells at baseline (p=0.0076; p=0.0133) and after activation with nano-PIC DC (p=0.0076; p=0.0133), respectively. Notably, higher levels of TRAIL were enriched in NKG2C+ cells from good compared to bad PLWH responders after nano-DC stimulation. Moreover, TRAIL was enriched in NKG2C+CD57- NK cells, compared to NK cells expressing CD57 (p=0.0391). In this regard, TRAIL negatively associated with expression of TIGIT in CS6dim CD16+ NK cells (p=0.0125; r=-0.55). By the other hand, p24+ CD4+ T cells from PLWH showed higher expression of the TRAIL ligand DR4 than p24- CD4+ T cells. Finally, cytotoxic NK function against HIV-1 infected p24+ CD4+ T cells was abrogated in presence of anti-TRAIL blocking mAbs, in contrast to anti-NKG2C and anti-NKG2D.

**Conclusion:** In conclusion, cytotoxic function of memory-like NKG2C+ CD57- NK cells associated with good functional elimination of HIV-1 reservoir CD4+ T cells is mediated by TRAIL.

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**444**  
**Single-Cell, Multi-Omics Analysis of SIV-Specific CD8 T-Cells Across Multiple Anatomical Sites**

**Jennifer Simpson**, Paul Schaughency, Justin Lack, Jason Brenchley

National Institutes of Health, Bethesda, MD, USA

**Background:** CD8+ T cells contribute to the antiviral response in simian immunodeficiency virus (SIV) infection in nonhuman primates. CD8+ T cells recognize viral epitopes via the T cell receptor (TCR). In previous studies, we examined the TCR repertoire of CD8+ T cells specific for a single SIV immunodominant epitope, Gag-CM9 (CTPYDINQM), throughout SIV infection. We identified tissue specific TCR sequences and TCRs shared by multiple anatomical sites. We now sought to evaluate if the tissue localization or TCR sequence of a CM9 specific CD8+ T cell corresponds with a transcriptional phenotype.

**Methods:** CM9 specific CD8+ T cells were sorted from blood, lymph nodes, spleen, and liver from SIV infected rhesus macaques. The cells were processed through the 10x Genomics single-cell sequencing protocol, creating a TCR amplified library and an RNA gene expression library for each cell. The sequenced libraries were demultiplexed and integrated using Cell Ranger and Seurat software. Clonotypes that reside within specific tissue specific and shared across multiple sites and clonotypes shared across multiple individuals (public) were identified.

**Results:** Clustering analysis on the transcriptional profiles of CM9 specific CD8+ T cells revealed no obvious clustering by animal, or the presence of a public TCR sequence and minimal clustering was observed by tissue. Cells with tissue specific TCRs were most numerous in the liver, CM9 specific CD8+ T cells with a tissue specific TCR had higher expression of the tissue resident marker gene ITGAV. Multidimensional scaling analysis of CM9 specific CD8+ T cell transcription within the same tissue showed distinct distances between cells with shared and tissue specific TCRs in the blood, lymph nodes and liver. Interestingly, most of the transcriptional variation was captured by the spleen, rather than CM9 specific CD8+ T cells from peripheral blood.

**Conclusion:** These data suggest that the tissue of origin of a CM9 specific CD8+ T cell, or the presence of a public TCR sequence does not associate with a distinct transcriptional profile across multiple anatomic sites. However, within the same tissue, whether a cell has a shared or tissue specific TCR sequence does appear to associate with a different transcriptional profile. Importantly, analysis of antigen specific CD8+ T cells from peripheral blood does not capture immunological phenomena occurring within tissues.

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**445**  
**Revealing the Role of Tissue-Resident NK Cells in HIV Infection Using Tissue Explant Models**

**David Perea**, Nerea Sanchez Gaona, Ana Gallego Cortés, Stefania Landolfi, Felix Pumarola, Nuria Ortiz, Ines Llanes, Juan Lorente, Vicenç Falco, Meritxell Genesca, Maria Bustin

1Vall d’Hebron Research Institute, Barcelona, Spain, 2Hospital Universitario de la Vall d’Hebron, Barcelona, Spain

**Background:** Tissue-resident NK cells participate in immune clearance of tumor and infections, yet their response against HIV infection may vary across tissue microenvironments. Here, we characterized NK cells by their memory-like and residency properties, and assessed their functionality in killing HIV-infected cells in relevant tissue models of HIV infection.

**Methods:** We obtained tissue resections from tonsils (n=38) and colon (n=32) from routine surgeries. Flow cytometry was used to quantify residency markers (CD49a, CD69, CD103), the memory-related marker NKG2C, and Killer-cell...
immunoglobulin-like receptors (KIRs) (KIR2DL1, S1, L2, L3, S2). Additionally, we measured natural cytotoxicity (CD107a, IFN-γ) after coculturing tissue cells with HLA-negative K562 cells in suspension, 2D coatings and 3D gels with collagen I and IV. Using tonsil and colon explants, we additionally characterized NK cells following ex vivo HIV(BaL) infection for 5-7 days.

**Results:** Both tissues shared similar NK cell residency patterns, predominantly CD16-CD69+. Among these, CD49a+CD103- and CD49a+CD103+/- subpopulations exhibited the highest and lowest proactivation levels, respectively (median % of CD107a+IFN-γ+ in tonsil: 70.60% vs 9.26%; colon: 77.90% vs 18.35%). However, robust natural cytotoxicity was exclusive to tonsillar NK cells, with CD16+CD49a- “adaptive” (KIR+HK2C+) cells showing a median 9.85-fold higher CD107a+IFN-γ+ cells upon stimulation. Of note, we observed that coculture on 2D coatings with collagen I or IV increased the IFN-γ in this subset, and K562 killing in 3D coating I was enhanced in both tissues (tonsil: 1.75-fold; gut: 1.37). After 5-7 days of HIV infection, tonsils exhibited 10% more KIRs expression on CD16+CD69+CD49a+CD103- NK cells (p=0.032), yet the presence of CD69+KIRs+ NK cells correlated with lower levels of HIV infection (Fig. 1A). Conversely, in the colon, the CD16+CD103+ population significantly expanded (1.27-fold, p=0.001), correlating with reduced HIV infection levels when expressing HK2C (p=0.017). Moreover, more CD16+CD69+CD49a+CD103+/- tonsillar NK cells were activated (% CD107a+IFN-γ+) after ex vivo HIV infection than in uninfected tissue explants, and CD16-CD103+ and multiple resident NK cells showed increased CD107a expression upon K562 stimulation post-HIV exposure (Fig. 1B).

**Conclusion:** This study underscores tissue-specific NK cell responses to HIV and their potential role in limiting HIV establishment in tissues, offering insights for future research and therapies.

**Results:** In the trial, early post-rebound, the frequency of IFN-γ+ HIV-specific CD8+ T cells (sum: Gag+Pol+Nef+Env) increased in those who exhibited control (median fold-change [FC] vs pre-rebound: 1.8x) but not in those who failed to control (FC 0.9x). Similarly, the frequency of proliferating (Ki67+) non-native CD8+ T cells increased robustly in controllers (FC 2.8x; 2.7 to 8.8%) but not in non-controllers (FC 1.2x; 2.8 to 3.3%). Higher %Ki67+ non-naive CD8+ T cells early post-rebound was associated with lower VL set point (r=-0.81, p<0.001). Similarly, post-AI in the second study, the fraction of proliferating CD8+ T cells (expressing HK2C) in the UI was higher in controllers vs non-controllers (3.6% vs 0.8%).

**Conclusion:** To our knowledge, these studies are the first to demonstrate a relationship between the early in vivo CD8+ T cell proliferative response to viral reactivation and HIV control post-AI. The results support continued focus on developing HIV cure strategies that enhance HIV-specific CD8+ T cell proliferative capacity.
Cannabis Inhalation Induces Significant DNA Methylation Changes Mediated by Host Response to HIV and Infection in PLHIV

Xun Jiang1, Elise M. Meeder1, Manoj K. Gupta1, Zhenhua Zhang2, Yang Li3, Mihai Netea2, Andre J. van der Ven4, Cheng-Jian Xu5

1Medizinische Hochschule Hannover, Hannover, Germany; 2Radboud University Medical Center, Nijmegen, Netherlands

Background: Cannabis use is prevalent among people living with HIV (PLHIV). Cannabis is mostly smoked leading to inhalation of the psychoactive tetrahydrocannabinol as well as reactive particles, toxins, and oxidants that may induce epigenetic changes and affect immune responses. We therefore analyzed the effect of cannabis on DNA methylation and immune function in PLHIV.

Methods: Participants were recruited from the 2000HIV study (NCT03994835), a Dutch multi-center study amongst virally suppressed PLHIV, separated into a discovery cohort (n=1512, 280 cannabis users) and a validation cohort (n=317, 47 cannabis users). Cannabis use and tobacco smoking were recorded through a self-reported questionnaire. Genome-wide DNA methylation data was obtained using the Illumina Infinium MethylationEPIC array. Plasma proteins were assessed by targeted proteomics (Olink Explore). PBMC ex vivo cytokine production capacity was measured upon bacterial, fungal, and viral stimulation. The differential DNA Methylation Sites (DMSs) were identified by an epigenome-wide association study, followed by integration with the proteomics, and cytokine secretion data from the same individuals.

Results: In the discovery cohort, 3741 cannabis-associated DMSs were identified, with 293 DMSs being replicated in the validation cohort. The most significant DMS is cg01940273 (p=1.3e-50) also strongly associated with tobacco smoking in 2000HIV and other studies. After accounting for tobacco smoking effects, 98% DMSs showed reduced significance but the methylation trend remained consistent and 81% DMSs were demethylated. Validated DMSs associated genes were enriched in nervous system development and leukocyte differentiation. The DMS-associated plasma proteins suggested that DMSs are associated with leukocyte differentiation and cytokine expression, such as IL6 upregulation and CXCL10 downregulation (Figure 1). The causal inference test suggested that cannabis-induced DMSs may modulate immune response in PLHIV, such as increased IL22 and decreased INF-γ production after PBMC stimulation.

Conclusion: Cannabis inhalation induces significant DNA methylation changes in PLHIV. Such modifications may lead to systematic changes in plasma (inflammatory) protein levels, and cytokine secretion of circulating immune cells. Downregulation of INF-γ and INF-γ-induced protein CXCL10 may influence host response to HIV and co-pathogens. Our data indicate that cannabis use needs to be recorded and corrected for reporting inflammation in PLHIV.

Cannabis-Induced DNA Methylation Changes Mediate Immune Response in PLHIV

Xun Jiang1, Elise M. Meeder1, Manoj K. Gupta1, Zhenhua Zhang2, Yang Li3, Mihai Netea2, Andre J. van der Ven4, Cheng-Jian Xu5

1Medizinische Hochschule Hannover, Hannover, Germany; 2Radboud University Medical Center, Nijmegen, Netherlands

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Conclusion: Cannabis inhalation induces significant DNA methylation changes in PLHIV. Such modifications may lead to systematic changes in plasma (inflammatory) protein levels, and cytokine secretion of circulating immune cells. Downregulation of INF-γ and INF-γ-induced protein CXCL10 may influence host response to HIV and co-pathogens. Our data indicate that cannabis use needs to be recorded and corrected for reporting inflammation in PLHIV.
Expression of HIV-1 icRNA sensing in HIV-1 infected macrophages and offer insight into potential targets for therapeutics to alleviate chronic immune activation in PWH.

**Results:** Though initiation of MAVS signaling can activate transcription factors, IRF3, IRF7, and IRF3, disruption of IRF5 expression had the highest impact on HIV-1 icRNA-induced type I IFN and IP-10 expression. Furthermore, attenuation of Traf6 (necessary for IRF5 ubiquitination and nuclear localization) expression, but not IkK-β, abrogated IP-10 production in macrophages. Interestingly, expression and nuclear localization of IRF5 was constitutively upregulated in older MDMs which translated to higher IFN-β and IP-10 expression, though infection establishment and HIV-1 icRNA expression was similar in MDMs from both younger and older donors.

**Conclusion:** Collectively, these results elucidate the mechanism of icRNA sensing in HIV-1 infected macrophages and offer insight into potential targets for therapeutics to alleviate chronic immune activation in PWH.

**451 IRF5 Mediates Persistent Inflammatory Responses in HIV-1-Infected Macrophages**

Sita Ramaswamy1, Jacob Berrigan1, Hisashi Akiyama2, Andrés Quiñones1, Alex Olson2, Yunfan Chen1, Yan Mei Liang1, Rahm Gammuluru2
1Boston University, Boston, MA, USA, 2Boston Medical Center, Boston, MA, USA

**Background:** People living with HIV (PWH) experience chronic inflammation, which can contribute to HIV-associated comorbidities. Long-lived HIV-1-infected macrophages are important mediators of chronic innate immune activation. Additive effects of aging and persistent HIV-1 infection can promote macrophage dysfunction, contributing to chronic inflammation, or "inflammaging". We previously reported that nuclear export and cytoplasmic expression of HIV-1 intron-containing RNA (icRNA) activates MAVS-dependent innate immune sensing and persistent type I IFN responses in macrophages. However, the signaling pathways downstream of HIV-1 icRNA sensing have not been fully elucidated. In this study, we show that IRF5 mediates type I IFN responses downstream of icRNA sensing in HIV-1 infected macrophages, and that persistent IRF5 activation in older PWH may contribute to inflammaging.

**Methods:** THP-1/PMA macrophages or monocyte-derived macrophages (MDMs) with selective abrogation of MAVS signalsome members, IRF3, IRF5, TRAF6, and IKK-β were infected with a single cycle HIV-1 reporter. MDMs were also differentiated from CD14+ monocytes derived from a primary patient cohort, stratified by age into younger (<35 y) and older (>50 y) donors. Cells were harvested at 2-3 days post infection and analyzed for infection establishment by flow cytometry, RT-qPCR for ISG expression, while cell-free supernatants were harvested for type I IFN and IP-10 production by biosay and ELISA, respectively. Infected macrophages were also fixed on coverslips for determining nuclear-cytoplasmic IRF5 localization using immunofluorescence microscopy.

**Results:** Blockade of MAVS signaling can activate transcription factors, IRF3, IRF7, and IRF5, disruption of IRF5 expression had the highest impact on HIV-1 icRNA-induced type I IFN and IP-10 expression. Furthermore, attenuation of Traf6 (necessary for IRF5 ubiquitination and nuclear localization) expression, but not IkK-β, abrogated IP-10 production in macrophages. Interestingly, expression and nuclear localization of IRF5 was constitutively upregulated in older MDMs which translated to higher IFN-β and IP-10 expression, though infection establishment and HIV-1 icRNA expression was similar in MDMs from both younger and older donors.

**Conclusion:** Collectively, these results elucidate the mechanism of icRNA sensing in HIV-1 infected macrophages and offer insight into potential targets for therapeutics to alleviate chronic immune activation in PWH.
453 Type I Interferon Drive Sustained Dominance of CCR5-Tropic Variants During HIV Infection In Vivo
Priya Pal, Sara Nicholson, Hongbo Gao, Liang Shan
Washington University at St Louis, St Louis, MO, USA

Background: A curious phenomenon is observed in people living with HIV (PLWH) who are not on antiretroviral therapy. Despite there being no difference in viral fitness between CXCR4 (X4)-tropic and CCR5 (R5)-tropic HIV viruses in vitro, R5-tropic viruses typically dominate in the early clinical stages. While R5-tropic viruses are selected during transmission events, it is surprising that X4-tropic viruses take years to emerge, if at all. CXCR4, the co-receptor for X4-tropic viruses, is expressed by the vast majority of CD4+ T cells while CCR5 is expressed by ~15% of total CD4+ T cells. In addition, it only takes a few amino acid changes to switch tropism. Even under maraviroc treatment, a CCR5 antagonist, X4-tropic viruses rarely emerge. Instead, resistant viruses arise that can bind to the maraviroc-bound CCR5, which requires generation of multiple simultaneous mutations. These studies suggest that X4 viruses are stably suppressed by the immune system in vivo beyond transmission. We aimed to understand the molecular mechanism of X4-specific immune control in vivo.

Methods: In vitro models are not suited to explore the mechanism of RS dominance. To better understand tropism specific immune control of HIV, an interferon-β receptor knockout (IFNAR-KO) human immune system was reconstituted in mice. Briefly, human CD34+ cells were transduced with Cas9 protein and guiding RNAs targeting the IFNAR coding gene or scrambled controls by electroporation before injection into 1-3 day old pups. Nine weeks post engraftment, these mice were infected with X4- or RS-tropic HIV isolates.

Results: In humanized mice engrafted with unmodified CD34+ cells, X4- and RS-tropic viruses replicate equally well when present alone. In contrast, when mice were infected with both viruses, there was a near complete suppression of X4 viral replication and sustained dominance of RS viral replication, recapitulating what is observed in patients. This restriction of X4 viral replication was lost in mice with an IFNAR-KO immune system. High levels of type 1 IFN production was observed in mice challenged with both X4- and RS-tropic viruses, which resulted in selective suppression of X4-tropic viruses. These results suggest that emergence of X4-tropic viruses post viral transmission is likely blocked by type 1 IFNs.

Conclusion: Our work reveals that X4-tropic viruses are selectively restricted by type I interferon (IFN) beyond the transmission event. We will explore how this restriction is lost in the later clinical stages.

454 Crosstalk Between TLR-8 and RLR Enhanced Antiviral Immunity Against Acute HIV-1 and PWH
Killian Vlaming1, John L. van Hamme2, Pien M. van Paassen2, Tanja M. Kaptein1, Karel A. van Dorn1, Peter Reiss1, Anneleen Verboom2, Casper Rokx2, Monique Nijhuis1, John L. van Hamme4, Nicola Cotugno3, Maria Grazia Lain3, Nicola Cotugno3, Teunis B. Geijtenbeek1
1Academic Medical Center, Amsterdam, Netherlands, 2Erasmus University Medical Center, Rotterdam, Netherlands, 3University Medical Center Utrecht, Utrecht, Netherlands, 4University of California San Francisco, San Francisco, CA, USA

Background: Achieving an HIV-1 cure requires both reactivation of the viral reservoir and antiviral immunity. In HIV negative individuals we showed that cross-talk between TLR8 and RLR enhances antiviral immunity by increasing production of IL12, IL27 and type I IFNs, promoting Th1 and Tfh differentiation and boosting CD8+ cytotoxicity. Targeting TLR8 and RLR could elicit antiviral immunity required in a HIV-1 cure strategy. We investigated whether this cross-talk persists in the context of HIV-1, in acute infected, treated, individuals as well as individuals who started ART in the chronic phase.

Methods: Four distinct participant groups were included: age matched HIV-negative (n=28), HIV-positive individuals treated in the chronic phase of infection (n=28) (ART initiated at CD4 <300), and individuals that initiated ART during the acute phase of infection (24-weeks (n=17) post-treatment initiation and three years (n=11) post-treatment initiation). PBMC were stimulated with TLR8 and RLR agonists alone or in combination for 24 hours. Immune responses; cytokine levels, type I IFN responses, and co-stimulatory molecules, were assessed.

Results: TLR8 agonist SGS6868 induced pro-inflammatory cytokines IL6 and IL12. Poly(I:C) and RLR agonist, induced IL27 in PBMC in all 4 groups. In HIV-negative individuals combination of the TLR8 agonist and RLR agonist resulted in a two-fold increase of IL12 and IL27, while IL6 secretion was decreased compared to single-stimulation. In the acutely treated individuals (24 weeks, 3 year post treatment initiation) cross-talk was largely preserved when comparing to HIV-negative individuals. However, notably, in individuals treated during chronic infection as compared to HIV negative individuals, TLR8/RLR cross-talk was impaired: the level of IL12 and IL27 was 2 to 1.5 fold lower, and IL6 reduction decreased from 1.9 to 1.2 fold.

Conclusion: Crosstalk between TLR8 and RLR is disrupted following chronic infection with HIV-1, initiation of ART in the acute phase of infection preserves this crosstalk to a large extent. Our data suggest that HIV-related monocyte dysfunction might underlie this lack of crosstalk, as monocytes respond to this form of TLR8 and RLR stimulation. Differences between treated acute and chronic infection suggest that this phenomenon is more pronounced during chronically treated infection. Deciphering these alterations in crosstalk is paramount for pioneering strategies that bolster immunity and eradicate reservoirs, especially in a chronic setting.

455 The Effect of HIV Infection at Pre-ART Initiation on the Early Innate Immune System
Vinh B. Dinh1, Lesley de Armas2, Rajendra Pahwa3, Suresh Pallikkuth4, Christine Deng1, Stefano Rinaldi4, Nicola Cotugno3, Paolo Palmia4, Nadia Stito4, Paula Vaz1, Maria Grazia Lain3, Savita Pahwa3
1University of Miami, Miami, FL, USA, 2Bambino Gesù Children’s Hospital, Rome, Italy, 3Instituto Nacional de Saúde, Maputo, Mozambique, 4Fundação Ariel Gásper Contra o SIDA Pediatría, Maputo, Mozambique

Background: Despite the advent of antiretroviral therapy (ART), perinatal HIV infection still occurs, mostly in low-income countries. The nature of the innate immune response to HIV infection in infants is relatively unknown, as it is an understanding of the effect of HIV replication on the development of the early innate immune system.

Methods: 68 perinatally HIV exposed infants from Maputo, Mozambique were diagnosed as infected (HEI, n=33) or not infected (HEU, n=35) at 1 month of age, prescribed ART for HEI and followed until 24 months. PBMC were isolated from blood at the clinical site and cryopreserved before being shipped to Miami. Flow cytometry was performed using a 27-color panel on PBMC to investigate the effect of HIV on innate immune cells (NK and monocytes). NK cell subsets were defined based on CD56 and CD16 expression on lineage negative lymphocytes. Monocytes were identified based on CD14 and CD16 expression on lineage negative and HLA-DR positive cells. These cell types were analyzed for markers of immune activation (IA), immune regulation, and trafficking. A flow cytometry based assay was implemented to investigate the killing potential and degranulation of NK cells utilizing a highly sensitive in vitro target (K562) and HIV-infected target cell line (HUT78/SF2) in a small subset of the cohort.

Results: At study entry pre-ART, NK cells in HEI infants exhibited an altered profile of activation, inhibition, and trafficking receptors suggestive of a more activated profile when compared to HEU such as increased activation (CD18) and trafficking (CCR5) with decreased inhibition (NKG2A). In addition, similar alterations were observed in the monocyte compartment but to a lesser extent. CCR2 on NK cells and intermediate monocytes were highly correlated with pre-ART viral load. NK cells of HEI had a similar cytotoxic capacity as HEU towards both cell lines but increased degranulation towards the K562 cell line. By 10 months of age, disturbances in subset distribution and markers of activation, inhibition, and trafficking were partially reversed in both viremic and aviremic infants.

Conclusion: The data suggests that NK cells and monocytes of HEI have an activated profile that appears to diminish with age. In addition, these cells are associated with viral replication at pre-ART initiation. These observations warrant further investigation on the impact of HIV on development of the innate immune system and the role of Intermediate monocytes and NK cells on viral replication and reservoir establishment.

456 Inflammatory Monocytes Increase During HIV-1 Treatment Interruption Before Detectable Viremia
Natalia de la Force1, Anna Farrell-Sherman1, Renan Valieris2, Steven G. Deeks3, Israel Tojal da Silva4, Timothy J. Henrich1, Lillian Cohn1
1Fred Hutchinson Cancer Center, Seattle, WA, USA, 2Ac Camargo Cancer Center, Sao Paulo, Brazil, 3University of California San Francisco, San Francisco, CA, USA

Background: When antiretroviral therapy (ART) is interrupted, most people living with HIV (PLWH) experience rapid viral rebound within a few weeks. We currently have no reliable non-viral biomarkers of imminent viral rebound. Rebound viremia is defined by circulating HIV RNA, but we hypothesize that low levels of virus replicating in tissues may create measurable biological changes in the immune landscape prior to detectable viremia. These changes may be...
most detectable directly preceding rebound viremia, when the viral load has not reached the limit of detection and is still under tentative immune control.

**Methods:** To investigate changes in the immune system indicative of the first signs of viral rebound, we performed two separate analyses on a cohort of donors with HIV-1 who participated in an observational analytical treatment interruption (ATI). We performed single cell RNAseq on peripheral blood mononuclear cells (PBMCs) from a subset of 10 donors and analyzed soluble protein levels in plasma using three Olink 92-plex panels on 19 donors. For the purposes of analysis, we compared three distinct timepoints: on-ART (before ATI), pre-rebound (during ATI, before detectable viremia), and post-rebound (detectable viremia). scRNAseq data was analyzed using the Seurat integration workflow. Pathway analysis using MSigDB hallmark gene sets collection was performed on scRNAseq data. Olink protein levels were log-normalized and assessed with a paired Wilcoxon test.

**Results:** In these PLWH, we found a significant increase in monocyte subsets prior to detectable viral rebound. Non-classical inflammatory CD14-CD16++ monocytes increased in proportion by 1.9% (p=0.008) while classical CD14++CD16- monocytes increased in proportion by 3.6% (p=0.054) (Fig. 1). Pathway analysis indicated enrichment for IFNy, IFNα, and inflammatory response pathways within monocyte subsets. Olink analysis indicated an increase in soluble inflammatory proteins post-rebound related to immune activation (PD-L2, TRAIL), cytokine signaling (TNF, LAG3, GZMB), and NFkB signaling (CXCL9, CXCL10, IL12).

**Conclusion:** Our data suggest that prior to detectable viremia, a subset of circulating monocytes activate and expand in response to viral rebound replication. These cells shift to a more inflammatory phenotype, indicating innate immune sensing of early viral rebound activity. This has the potential to be a non-viral biomarker of imminent viral rebound.

![CD14+ Monocytes vs CD16+ Monocytes](image)

**Intact Proviruses Persist in Expressed Genes in People With Non-Suppressible HIV on Long-Term ART**

Joshua A. Gluck1, Sean Patro2, Elias K. Halvas3, Kevin Joseph1, Nathan McKenna1, Shuang Guo1, Shadab Parvez1, Jason W. Rausch1, Xiaolin Wu1, John M. Coffin1, John W. Mellors1, Stephen Hughes2, Mary F. Kearney1

1National Cancer Institute, Frederick, MD, USA, 2Celera Bioscience Research, Inc, Frederick, MD, USA, 3University of Pittsburgh, Pittsburgh, PA, USA, 4Tufts University, Boston, MA, USA

**Background:** The persistence of replication-competent proviruses during ART is a key barrier to an HIV cure. A previous study identified 3 cell clones carrying sequence-intact HIV proviruses increased in size, one decreased, and seven did not change significantly over 1-4 years.

**Results:** We isolated 9 Jurkat T cell clones, each with HIV provirus (NL4-3-d6-dE-dsGFP) stably integrated into (a) introns of an actively transcribed gene in the same orientation (VAV1, RAP1B, SPECC1), (b) introns of an actively transcribed gene in the opposite orientation (INPPL1, FKBP1, KLF12, EEZF, and c) intergenic regions. Using CRISPR-mediated activation or inhibition of HIV expression remains unsettled. We postulated that host promoter activation increases local chromatin accessibility to transcription factors and increases HIV expression.

**Methods:** We isolated DJurkat T cell clones, each with HIV provirus (NL4-3-d6-dE-dsGFP) stably integrated into (a) introns of an actively transcribed gene in the same orientation (VAV1, RAP1B, SPECC1), (b) introns of an actively transcribed gene in the opposite orientation (INPPL1, FKBP1, KLF12, EEZF, and c) intergenic regions. Using CRISPR-mediated activation or inhibition of HIV expression, we postulated that host promoter activation increases HIV gene expression using ATAC-seq, RNASeq, and qRT-PCR. We assessed differential gene expression and chromatin accessibility using edGER and chromatin footprints with TOBIAS.

**Results:** Activation of the host promoter dominantly decreased HIV gene expression in 4 out of 7 cell line clones: SPECC1, KLF12, EEZF (each FDR<0.05), and VAV1 (FDR<0.05), in both orientations of HIV integration. A smaller trend was seen with RAP1B (FDR=0.07). For the cell line clone in which HIV integrated 7kb from the host promoter, activation of the host promoter INPPL1 increased HIV expression. The two clones harboring a provirus in intergenic regions expressed substantial proviral RNA despite lower chromatin accessibility. We next examined whether host promoter activity changes chromatin accessibility and transcription factor binding to the HIV LTR. Activating the host promoter of VAV1, RAP1B, SPECC1, and EEZF significantly reduced chromatin accessibility of the downstream HIV 5’ LTR (FDR<0.05; KLF12 n.s.) and displaced HIV transcription factors. In contrast, activating host promoter INPPL1 increased HIV LTR chromatin accessibility.

**Conclusion:** We identified transcriptional interference at the HIV integration site. Activation of host genes harboring an HIV integration site displaces transcription factors bound to the HIV 5’ LTR and inhibits HIV expression. In vivo studies to understand or modulate latency should account for transcriptional interference.
Role of HIV Integration Site on Clonal Expansion of Infected Cells and Maintenance of Latency

Virender K. Pat1, Frauke Muecksch2, Ali Danesh3, Marie Cani3, Tan T. Huy3, Theodora Hatzioannou2, R. Brad Jones4, Guinevere Q. Lee5, Paul D. Bieniasz6

1The Rockefeller University, New York, NY, USA, 2Heidelberg University, Heidelberg, Germany, 3Weill Cornell Medicine, New York, NY, USA

Background: Latent reservoirs of HIV-1 are the major barrier to achieving a cure. Because latently infected cells are rare in humans there is a paucity of understanding of how latent reservoirs survive and expand, escape immune clearance, and reactivate upon ART cessation. The site of HIV-1 proviral DNA integration into the host genome could (i) determine the transcription status of the provirus and (ii) influence the expansion of infected clones during ART. In this study we are interested in the characterizing proviral integration landscape associated with clonal expansion and latency of HIV-1 infected cells in an in vivo model.

Methods: Primary memory CD4+ T cells isolated from healthy human donors were infected with dual reporter tagged, defective HIV-1 to generate large populations (millions) of human memory CD4+ T cells each presumptively carrying a single reporter provirus at a distinct integration site. These cells were profiled inside NSG mice and analyzed for proviral transcriptional dynamics over a period of time. HIV-1 integration sites were characterized by PCR-based amplification of host-viral junction and next-generation sequencing.

Results: Our preliminary analysis of HIV-1 integration sites in these long-term persistent HIV-1 infected cells led to the identification of 1777 unique integration sites (UIS). Consistent with previous reports, HIV-1 integration was favored in genic regions (60%) of human chromosomes as compared to non-genic regions (40%). Genic HIV-1 integrations were primarily in introns (94%). We found 84 genes that harbored HIV-1 integrations in multiple different clones and in most cases these integrations were clustered within a single intron, suggesting possible hotspots within a gene for integration or for clone survival. Two different mice had clones with HIV-1 integrants in the same 25 genes suggesting a growth or survival advantage for clones harboring HIV-1 integration in these genes. Overall, 641 UIS were associated with expanded clones persisting in vivo and clonally expanded cells were more likely to have HIV-1 integration inside genes than cells where clonal expansion was not detected. The genes in which HIV-1 integration was associated with clonal expansion belonged to pathways with roles in cell component biogenesis, mitosis, regulation of cell cycle, and chromatin remodeling.

Conclusion: Clonal expansion of HIV-1 infected cells in this model system is associated with proviral integration in a set of genes which provide selective growth advantage in vivo.

460 HIV Integration Site Features Associated With Persistence of Infected Cells Under CD8 Pressure

Noemi L. Linden1, Alexander McFarland2, Ali Danesh3, John Everett2, Scott Sherrill-Mix4, Carole Lee5, Chasson J. Brumme2, Dennis Copertino6, Karina Brumme2, N. Hayzana Miller3, Tan T. Huy3, Asile Roche7, Jared Weiler1, Frederic D. Bushman8, R. Brad Jones4

1Weill Cornell Medicine, New York, NY, USA, 2University of Pennsylvania, Philadelphia, PA, USA, 3Michigan State University, East Lansing, MI, USA, 4British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada, 5Simon Fraser University, Vancouver, Canada

Background: HIV integration sites (IS) in elite controllers and after long-term ART are highly clonal and skewed towards transcriptionally silent genomic loci. The mechanisms contributing to this IS landscape remain unclear, hampering the design of cure therapeutics. Using a participant-derived xenograft mouse model, we observed that CD8+ T-cell pressure was sufficient to recapitulate key features of this IS profile, including enrichment for large clones and integrations distal from genes. Here, we expand our dataset and assess whether prolonged elite controller CD8+ T-cell pressure is associated with integrations into specific gene pathways.

Methods: NSG mice were infected with memory CD4+ T cells from two HLA-B27+ HIV male elites and controller and infected with HIVVRCSF to test the effect of autologous memory CD8+ T-cell engraftment on the proviral landscape. T cell responses and HIV viral loads were monitored weekly by flow cytometry with a Gag-KK10 tetramer and by qPCR. At week 8, splenic DNA was subjected to linker-mediated PCR for IS sequencing. IS were identified with the AAVerger software pipeline. We performed Reactome pathway analysis on 6,402 and 12,406 unique IS from 18 CD8+ and 9 CD8- mice respectively. A Bayesian mixture model was developed to incorporate different IS associated characteristics of CD8+ T-cell pressure.

Results: The top 10% of expanded clones harbored integrations in recurrent integration genes previously identified in clinical studies, such as STAT5B, BACH2, MKL1, as well as genes observed in rare HIV-related tumors, including STAT3 and LCK. In the absence of CD8+ T cell pressure, HIV integrations were enriched in genes involved in cell cycle and cellular signaling pathways (p.adj<1e-6). In the presence of CD8+ T-cells, integrations frequently targeted genes involved in chromatin organization, miRNA processing, modification, and trafficking, as well as SUMOylation (p.adj<1e-9). The Bayesian model determined a CD8+ T-cell selection score of each mouse, thus functioning as a tool for quantifying CD8+ T-cell selective pressure, and highlighted donality as a prominent characteristic associated with CD8+ T-cell selection.

Conclusion: Our findings support a role for CD8+ T-cell pressure in selecting for particular clones of infected cells. Enrichments in gene pathways may reflect accessibility for integration, but also raises the possibility of CD8+ T-cell selection for insertional mutations events that might modulate the transcription of genes involved in HIV expression.

461 HIV Proviruses Among CMV- Reactive Cells Are Abundant, Defective, and Polyclonal After 4 Years of ART

Filippo Dragoni1, Mauria Manion1, Hao Zhang2, Angelica Camile-Contreras3, Frank Maldarelli4, Irini Sereti4, Francesco R. Simonetti5

1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 4National Cancer Institute, Frederick, MD, USA

Background: Since clonal expansion plays a major role in the maintenance of HIV-infected CD4+ T cells, antigen exposure and immune reconstitution can affect reservoir dynamics. Chronic CMV infection is characterized by percolating antigen expression and progressive inflammatory T-cell memory. Although HIV-infected, CMV-reactive cells are often dominated by large clones, which factors lead to their selection remain unclear. We assessed the contribution of immune reconstitution, CMV-related disease, and CMV-DNAemia to HIV persistence.

Methods: We studied 16 participants living with HIV and CMV, enrolled in two NIAID clinical trials. All had a CD4 nadir <50cells/µL. CMV-driven immune reconstitution inflammatory syndrome (IRIS) and end-organ disease were present in 3 and 3 participants, respectively. PBMCs from week 192 on ART were CD8-depleted and stimulated with CMV antigens. Reactive cells (positive for CD69/CD154/CD137) were plated–sorted at limiting dilution and subjected to whole genome amplification. HIV-1 wells were tested for LTR, IPDA, proviral sequencing, and integration site analysis. Bulk TCRseq was performed on cells reactive to CMV and CD3/CD28.

Results: CMV DNAemia was detected in 11/16 participants but decreased below LOD upon CD4 recovery. Only 4/16 participants reached CD4 counts >500 cells/µL by week 192. CMV-reactive cells were highly variable but detectable in 15/16 participants (median 2%, range 0.6-18%). We observed a high frequency of HIV DNA among CMV-reactive cells, with a median of 4328 (2446-5654) proviruses/million cells. IPDA showed 2-fold lower total DNA (p=0.0006) and rare intact genomes, suggesting that the majority of proviruses are highly defective or solo LTR. Based on gag and env sequencing, we detected only a few identical proviruses in most individuals. Among those expanded, we found proviruses in loci linked to heterochromatin (ZNF84) and insertional mutagenesis (STAT5B). TCR sequencing revealed marked clonality of CMV-reactive cells compared to the CD3/CD28 control (p<0.0001). Across all analyses, we detected no correlation with CMV DNAemia, IRIS, or CMV disease.

Conclusion: We observed high infection frequency among CMV-reactive cells regardless of immune reconstitution and CMV-DNAemia. The discrepancy in clonality between total CMV-responding cells and those infected suggests a preferential expansion of uninfected cells, likely selected during ART, while older infected clones may require longer time to be substantially shaped by proliferation.

462 Differential Gene-Specific Selection Pressures on HIV-1 Defective Proviruses During ART

Thuy Nguyen1, Mary-Elizabeth Zipparo2, Lindsey Adams1, Annemarie Glasey3, Ulisses Santamaria4, Catherine A Rehm5, Jessica Earhart5, Wei Shao6, Chuen-Yen Lau5, Frank Maldarelli7

1National Cancer Institute, Frederick, MD, USA, 2Equus Biomedical Research, Inc, Frederick, MD, USA, 3National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 4National Institutes of Health, Bethesda, MD, USA, 5National Cancer Institute, Bethesda, MD, USA

Background: The error-prone nature of HIV-1 replication and host factors generate defective genomes. Defective proviruses persist, can be transcribed,
and translated contributing to HIV-1 pathogenesis. During antiretroviral therapy (ART), most defective proviruses contain large deletions and hypermutations precluding the investigation of gene-specific selection pressures. We studied selection pressures on whole HIV-1 genomes and subgenomes of >7kb near-full length (NFL) defective proviruses prior to following ART.

**Methods:** Blood cells were collected from 11 HIV-1 suppressed participants prior to and at multiple timepoints on ART (4-20 years). We performed single genome sequencing of >7kb proviruses and identified defective proviruses with stop codons, insertions/deletions or hypermutations. We collapsed identical sequences and compared the whole genomic and subgenomic characteristics of defective proviruses from pretherapy to ART. We predicted the Major Histocompatibility (MHC) class I binding, immunogenecity, and sensitivity to Broadly Neutralizing Antibodies (bNabs)

**Results:** We obtained 705 NFL defective sequences. Proportions of hypermutant proviruses were not significantly different prior (median 38.38%) to following ART (26.86%). We observed significant selection for proviruses with defects on major spliced donor/packaging signal (MSD/PSI) (33.33% prior to 84.44% on ART) and gag (40% to 75%) but against proviruses with defects on env (66.67% to 21.25%) (p-values: 0.001-0.006). However, Rev-response elements (RRE) remained very conserved during ART; a median (interquartile range) of 0 (0-5.8%) proviruses carried RRE defects. Patterns of gag defects mainly involved start codon or deletion while deletion was the only defect pattern on env. HLA-associated mutations emerged or significantly shifted in env of >711 participants, but these mutations did not cause changes in the predicted MHC binding affinity or immunogenecity. Significant changes in predicted bNabs sensitivity were observed for env of >711 participants.

**Conclusion:** NFL defective proviruses are subjected to significant differential selection pressures on MSD/PSI, gag and env under ART. Gag defects are likely linked to deletions on MSD/PSI suggesting the selection for proviruses with impaired replication and infectivity. Selection against deletions on env is possibly linked to HLA escape mechanism but did not lead to significant changes in MHC binding or immunogenecity suggesting the possible involvement of other immune and viral factors.

In-Depth Proviral Sequence Analysis Reveals Conserved Reservoir Landscape Composition Across Group M

Hannah J. MacLeod1, Elizabeth A. Ferrer2, Hanna R. Marks3, Mian Cai4, Albine Martin5, Gregory M. Laird6

1Accel Prev Diagnostics, Baltimore, MD, USA; 2Harvard University, Cambridge, MA, USA; 3University of California Los Angeles, Los Angeles, CA, USA

**Background:** The biology of HIV-1 replication and epidemiology of transmission led to a diverse landscape of viral subtypes heterogeneously distributed across the globe. The majority of people with HIV-1 (PWH) are infected with non-subtype B virus, yet the landscape of persistent HIV-1 is understudied outside of subtype B. In-depth analysis of persistent HIV-1 in PWH across subtypes is essential to globalize HIV-1 cure research and clinical trials. Critically, such analysis is also foundational to the design and validation of molecular assays for latent HIV-1, such as a cross-Group M IPDA.

**Methods:** We performed an extensive proviral sequencing campaign on samples from hundreds of PWH on ART with broad geographic and Group M coverage. We optimized and employed a new semi-automated, high-throughput, near-full length single-genome PCR (NFL-PCR) workflow to generate proviral amplicons paired with improved pipelines for proviral assembly, alignment, and defect mapping. Proviral quantity and landscape composition were compared between subtypes and CRFs across Group M.

**Results:** Our analysis revealed highly consistent proviral landscapes across Group M. In all subtypes, the majority of proviruses analyzed harbored fatal deletions, which on average spanned half the genome and most frequently impacted the env gene. A subset of proviruses were extensively hypermutated in all subtypes. Initial sliding window analysis of deletion position and frequency suggests comparable IPDA proviral discrimination across subtypes using psi and env targets. Optimization of NFL-PCR resulted in significantly increased overall provirus recovery efficiency, and genetically intact proviruses were quantified at a higher frequency in all subtypes (including B) compared with other reports.

**Conclusion:** The consistency in HIV-1 provirus deletion frequency and position across Group M suggests that conserved mechanisms drive formation of defective proviruses. The dominance of env deletions has important implications for antibody-mediated reservoir immune selection and for global deployment of env-targeting therapeutics. Our improvements to long-range proviral PCR reduce the bias against intact proviruses, resulting in a more accurate analysis of proviral landscape composition in PWH on ART, and will enable comparison of short- and long-range PCR-based reservoir quantification assays. Importantly, our results support the feasibility of a unified IPDA version 2 design with robust performance across Group M for global use.
using HIVseqinR. Viral subtyping was performed with RIP 3.0. Viral tropism was inferred using V3-loop sequences with a subtype-specific algorithm PhenoSeq.

**Results:** We obtained 697 viral DNA genomes, of which 320 contained full-length Env (247 intact and 73 defective) representing all 24 donors. Intact viral DNA genomes were detected in 23 out of 24 donors; V3-loop analysis revealed that 61% donors were entirely R5-tropic (n=13 subtype C, n=1 A1/K), 26% donors were entirely X4-tropic (n=6, all subtype C), and 13% donors had a mix of R5- and X4-tropic viral DNA genomes (n=3 all subtype C). Tropism remained stable in 82% (14/17) of donors who had longitudinal data, including six who initiated antiretroviral therapy within 1-2 days post-detection, as well as eight who initiated treatment during chronic infection due to positive treatment interruptions and remained virologic rebound during treatment interruptions.

**Conclusion:** We detected an unexpected high prevalence of X4-tropic archived intact HIV-1 DNA in acute infections in FRESH by PhenoSeq, confirmed by geno2pheno (coreceptor) and C-PSSM (results not shown). Given that previous studies have associated subtype C and acute infections with mostly R5-tropic variants, our results suggest phenotypic studies are needed to further assess the extent and roles of archived X4-tropic variants in intact HIV-1 DNA genomes, which may have implications in the types of cells that would fuel virologic rebound during treatment interruptions.

### 466 Multimomics Profiling of HIV-Transcribing Cells in People With HIV on Suppressive ART

**Julie Frouard**, Xiaoyu Luo, Sushama Telwatte, Douglas Amerson, Jason Neidlemann, Kailin Yin, Antoine Chaillon, Rebecca Hohn, Steven G. Deeks, Sara Gianella Weibel, Davey M. Smith, Sulggi A. Lee, Phyllis Thien, Steven A. Yuki, Nadia R. Roan

**University of California San Francisco, San Francisco, CA, USA, *University of California San Francisco, San Francisco, CA, USA, **University of California San Diego, La Jolla, CA, USA**

**Background:** HIV reservoir cells actively transcribing HIV persist despite ART, but remain poorly characterized since standard single cell (sc)RNAseq approaches inefficiently identify these cells. In this study, we devised a comprehensive scRNAseq-based approach that increases identification of HIV-transcribing cells, enabling a deep characterization of these cells.

**Methods:** We developed HIV-seq, an approach whereby HIV primers targeting multiple regions of the HIV genome are added during scRNAseq library preparation to increase our ability to detect HIV RNA+ cells from people with HIV (PWH) under suppressive ART. We paired HIV-seq with a new bioinformatics pipeline, named 2-Step HIV Alignment, to further increase our ability to identify HIV transcripts from scRNAseq datasets. This entailed realigning initially unmapped reads to 1) a subtype B consensus sequence under reduced stringency conditions, and 2) participant-specific autologous viral sequences. HIV-seq was combined with 2-Step HIV Alignment were implemented in the context of multi-omic single-cell sequencing analyses where transcriptomic (scRNAseq), surface phenotypic (CITeseq), and clonal expansion (TCR analysis) data were simultaneously captured on the same cells. A total of 18 PWH under ART were recruited for this study, and 4 of them also provided virologic samples.

**Results:** Using this combined approach, we were able to increase by up to 40% our ability to detect HIV RNA+ cells from PWH. HIV RNA+ cells were identified from all blood samples and 7 tissue sites (spleen, lymph nodes, ileum, colon, rectum, endometrium, and endocarditis) of 15 donors on suppressive ART. The host transcriptome of these HIV RNA+ cells differed in a tissue site-dependent manner, and included Th17 and Trm cells. Unlike HIV RNA− cells from viromic individuals, those from suppressed individuals did not preferentially exhibit a cytolytic phenotype characterized by higher expression levels of granzymes, perforin, and granulysin. Shared clonotypes of HIV RNA+ cells (expressing the same TCR) were found in multiple tissue compartments; these cells showed distinct surface phenotypes and transcriptional profiles characteristic of their tissue site of residence.

**Conclusion:** Our study presents new tools that improve detection of HIV RNA+ cells at the single-cell level, enabling us to establish an atlas of HIV-transcribing cells from PWH on suppressive ART. HIV-transcribing cells clonally expand and disseminate to multiple tissue sites where they adopt tissue site-specific features.

### 467 HIV-1 Transcriptional Activity Is Largely Driven by Defective Proviruses

**Mareva Delporte**, Ery E. Blomme, Evelien De Smet, Maxime Verschoore, Marie-Angélique De Scheerder, Sofie Rutsaert, Sarah Gerlo, Wim Tysvreck, Linos Vandekerckhove

*Ghent University, Ghent, Belgium, *Ghent University Hospital, Ghent, Belgium

**Background:** The majority of cells that are latently infected with HIV-1 do not produce viral RNA, making it difficult for the immune system and current antiretroviral therapy (ART) to target them. To study the underlying mechanisms governing latent HIV-1 infection, a panel of reverse transcription droplet digital polymerase chain reaction (RT-ddPCR) assays specific for different HIV-1 transcripts has been described that define distinct blocks to transcription.

**Methods:** We designed the Rainbow transcriptional HIV-1 DNA 4+plex digital PCR assay, compatible with the QiAacuity 5-plex system, to quantify elongated (long LTR), unspliced (pol), multiple-spliced (Tat-Rev) and completed transcripts (polyA). To evaluate technical performance, a 2-fold dilution curve was prepared containing J-Lat copy DNA (cDNA), ranging from 0.005 to 2.5 ng input, and measured in 20 replicates. This assay was then applied to samples from 40 people living with HIV (PLWH) on ART. Additionally, the HIV-1 DNA reservoir was quantified by the Rainbow proviral HIV-1 DNA assay. Intactness levels were defined by two target regions: psi and env (IPDA), and by four target regions: psi, env, gag and pol (DAPCR). Levels of defective proviruses were measured by the difference between total and intact HIV-1 DNA levels (DAPCR).

**Results:** Linear quantification was observed for each HIV transcript (slope: 0.98). The Limit of Detection (LoD) was <10 copies/well for all HIV-1 RNA transcripts, except poly A (average: 11.1 copies/well, range: 5.6-24). The Limit of Blank (LoB) was calculated by using 90 negative template control (NTC) samples, resulting in an LoB of 1 copy/well or less in long LTR, pol and Tat-Rev. The LoB of polyA was 15.75 copies/well. In samples from 40 PLWH, HIV-1 RNA levels were compared to total and intact HIV-1 DNA levels (Figure 1). We found that intactness levels by IPDA correlated better with HIV-1 RNA transcripts than intactness levels by DAPCR. Interestingly, defective proviruses showed a stronger correlation with HIV-1 RNA transcripts compared to intact proviruses.

**Conclusion:** The Rainbow proviral HIV-1 DNA and transcriptional HIV-1 RNA assay allow us to map the characteristics of the viral reservoir by using a minimal amount of sample. This analysis resulted in a strong correlation between defective proviruses and HIV-1 RNA transcripts, suggesting that the HIV transcriptional activity is largely driven by defective proviruses.

### 468 Dynamics of Different Proviruses and HIV Transcripts After Acute ART Treatment

**Julie Janssens**, Sun Jin Kim, Adam Wdroychowski, Gayatri N. Kadiyala, Satish K. Pillai, Timothy J. Henrich, Nadia R. Roan, Steven G. Deeks, Sulggi A. Lee, Steven A. Yuki

*San Francisco VA Medical Center, San Francisco, CA, USA, *University of California San Francisco, San Francisco, CA, USA, *Gladiadite Institute of Virology and Immunology, San Francisco, CA, USA

**Background:** Different subsets of HIV-transcribing cells may contribute to immune activation and rebound. We investigated the dynamics of proviruses and different HIV transcripts after initiation of ART during acute infection. We hypothesized that completed, multiply spiked, and intact HIV RNA will decay at a faster rate than initiated, 5’ elongated, or defective HIV RNA.

**Methods:** CD4+ T cells were isolated from blood before ART (T1) and 6 mo (T2) ± 1 year after suppressive ART from 5 PWH (Treat Acute Cohort). U3-US, TAR, R-US/Gag and Pol HIV DNA regions, as well as 5’ defective (Psi−RRE−), 3’ defective (Psi+RRE+), and intact (Psi+RRE+) proviruses, were measured by ddPCR. HIV transcripts, including total initiated (TAR), 5’ elongated (R-US/Gag), mid-transcribed (Pol, unspliced), completed (U3-polyspliced), multiply spiked (Tat-Rev), 5’ defective, 3’ defective, and intact HIV RNA, were measured by RT-ddPCR. We also calculated ratios of each HIV RNA to the corresponding HIV DNA.
DNA (to account for proviral mutations) and ratios of one HIV RNA to another (to measure blocks to transcription).

**Results:** In untreated infection, we observed an excess of initiated over 5' elongated HIV RNA (median 5' elongated/initiated=0.19; P=0.03) but no significant difference between levels of 5' elongated and completed HIV transcripts. ART induced progressive reductions in initiated (median T1/T2=11; P=0.06), 5' elongated (84; P=0.03), mid-transcribed (143; P=0.03) and completed (503; P=0.03) HIV transcripts. These trends persisted after normalization to the corresponding HIV DNA. Completed transcripts decayed faster than initiated (P=0.03) or 5' elongated transcripts (P=0.03). The ratio of completed/5' elongated HIV RNA was lower at T2 than T1 (P=0.03). ART also reduced intact, 3' defective, and 5' defective HIV RNA (P<0.05 for all). Intact transcripts tended to decay faster than PSI+ RRE- HIV RNA (P=0.06). After normalizing to the corresponding HIV DNA, ART reduced PSI+ RRE- HIV RNA/DNA (median T1/T2=11.3; P=0.03) and tended to reduce intact HIV RNA/DNA (376.9; P=0.09).

**Conclusion:** Although ART does not target HIV transcription, the pattern of HIV transcriptional processivity differed between untreated infection (less of a block to completion) and early ART suppression. ART reduced completed HIV RNA more than initiated or 5' elongated HIV RNA, and tended to reduce intact HIV RNA more than 3' defective HIV RNA. These findings suggest distinct clearance of cells depending upon HIV RNA processivity and absence of proviral mutations.

469 Multimics of Detectable vs Undetectable Monocyte Cell-Associated HIV RNA During Acute HIV

**Michael J. Corley,1 Ivo Sahbandar,1 Philip Chan,1 Alina P. Pangi, Nittaya Phanuphak,1 Carlo P. Sacdalan,1 Sandhya Vasan,1 Lydie Trautmann,1 Serena S. Spudich,1 Lishomwa Ndhlovu,2 for the SEARCH101/RV524 Study Group

**Well Cornell Medicine, New York, NY, USA, 1Jular University, New Haven, CT, USA, 2SEARCH, Bangkok, Thailand, Henry M Jackson Foundation, Bethesda, MD, USA**

**Background:** Monocytes play a significant role in the early immune response during acute HIV infection (AHI), and the extent myeloid cell dysregulation has implications for long-term central nervous system (CNS) outcomes. We hypothesized that monocytes carrying HIV RNA would show increased transcriptional dysregulation during AHI.

**Methods:** We isolated ultra-high purity monocytes from 25-40 million PBMC aliquots of 17 participants in the Thai RV254/SEARCH010 AHI cohort obtained prior to ART during AHI (Fiebig I-V) to measure genome-wide transcriptome expression and epigenetic profiles and assess the detection of cell-associated (CA-) HIV RNA. Mann Whitney and T tests examined demographic differences between those with detectable and undetectable CA-HIV RNA. Differential expression analyses compared participants with detectable versus undetectable detectable HIV RNA using an FDR adjusted P value.

**Results:** Participants had a median age of 26 yrs, median log plasma viral load of 5.55 copies/mL (IQR: 4.87-6.51), CD4+ T cell count of 451 cells/mm³ (324.5-644), and CD4/CD8 ratio of 0.52 (0.37-1.09). 11/17 participants had detectable CSF HIV RNA levels in supernatant. 41% were in Fiebig stages I/II, and 59% in stages III-V. 8/17 (47%) had detectable monocyte HIV CA-RNA. Those with detectable monocyte HIV RNA trended higher in plasma viral load (6.17 vs 4.79; p=0.05) and lower in CD4/CD8 ratio (0.52 vs 1.02; p=0.06), but there was no difference in CD4 count or Fiebig stage between groups. 70 genes were detectable versus undetectable monocyte CA-HIV RNA. Differential expression analyses compared participants with detectable versus undetectable detectable HIV RNA using an FDR adjusted P value.

**Conclusion:** Participants with detectable ART within two years of HIV acquisition had lower hazard of viral blips. Further research to characterize the clinical implications of blips and how they relate to HIV reservoir dynamics is ongoing. HIV reservoir plasticity may extend beyond the period of acute HIV.

470 Antiretroviral Therapy Within 2 Years of HIV Acquisition Is Associated With Fewer Viral Blips

**Trevor A. Crowell,1 Hsing-Chuan Hsieh,1 Kun Wang,1 Xiuping Chu,4 Britt Gayle,1 Catherine M. Berjohan,1 Jason M. Blaylock,2 Joseph M. Yabes,3 Derek T. Larson,1 Robert J. O’Connell,1 Anuradha Ganeshan,1 Brian K. Agan,‡ for the Infectious Disease Clinical Research Program (IDCRP) HIV Working Group

1US Military HIV Research Program, Silver Spring, MD, USA, 2Uniformed Services University of the Health Sciences, Bethesda, MD, USA, 3US Military HIV Research Program, Bethesda, MD, USA, 4Naval Medical Center San Diego, San Diego, CA, USA, 5 Brooke Army Medical Center, San Antonio, TX, USA**

**Background:** Viral blips may represent bursts of HIV replication or clonal expansion from reservoirs. Antiretroviral therapy (ART) started within days of HIV acquisition may limit reservoirs, thereby decreasing blips, but is uncommon. We evaluated the impact of ART initiation within months to years after HIV acquisition on the occurrence of viral blips.

**Methods:** The ongoing U.S. Military HIV Natural History Study enrolls adult U.S. Department of Defense beneficiaries with HIV. HIV RNA results and ART medications are extracted from centralized electronic medical records. These analyses included participants who had an estimated HIV seroconversion date (midpoint of documented negative and positive test dates), achieved viral suppression (<400 copies/mL within 1 year after starting ART, and had at least 3 HIV RNA measurements after achieving VS. A blip was any HIV RNA 401-1000 copies/mL immediately preceded and followed by HIV RNA ≤400 copies/mL without a change in ART. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for factors potentially associated with the time from VS to first viral blip.

**Results:** From January 1996 to December 2022, 1,413 participants on suppressive ART met inclusion criteria for these analyses. Their median age at HIV diagnosis was 29.2 years (interquartile range 24.9-35.4) and a majority were males (96.3%). Viral blips were observed in 88 (6.2%) participants. Of these, 68 (77.3%) had a single blip, 13 (14.8%) had two blips, four (4.5%) had three blips, and three (3.4%) had four blips. The overall incidence of blips was 1.2 blips per 100 person-years (95% CI 1.0-1.4) and blip incidence decreased over time (Panel A). ART initiation within 24 months of estimated HIV acquisition was associated with decreased viral hazard of viral blips as compared to ART initiation after more than 24 months (0-6 months HR 0.32 [95% CI 0.20-0.52]; 6-12 months HR 0.48 [95% CI 0.35-0.67]; 12-24 months HR 0.52 [95% CI 0.40-0.69]; controlling for age, sex, race, time-updated ART regimen, time-updated CD4, HIV RNA at ART initiation, ART adherence (proportion of days covered >90%), history of hepatitis B, and history of hepatitis C; unadjusted in Panel B).

**Conclusion:** Participants who initiated ART within two years of HIV acquisition had lower hazard of blips. Further research to characterize the clinical implications of blips and how they relate to HIV reservoir dynamics is ongoing. HIV reservoir plasticity may extend beyond the period of acute HIV.
471 Reduced HIV RNA Transcription During Long-Term ART Is Associated With Increased Ki67+ CD4+ T-Cells


University of California San Diego, La Jolla, CA, USA, *National Institute of Child Health and Human Development, Bethesda, MD, USA, *Tufts University, Boston, MA, USA, *Brown University, Providence, RI, USA, *Brigham and Women's Hospital, Boston, MA, USA

Background: Sex-based differences affect HIV infection and immune responses. We investigate longitudinal T cell phenotypes and their association to HIV reservoir in men and women during long term suppressive ART.

Methods: From the AIDS Clinical Trials Group Longitudinal Randomized Trials, we identified 52 cisgender women and 29 age-matched men. Participants were between the ages of 40-53 at time of ART initiation and virally suppressed (<20c/ml) throughout the entire study period (318 timepoints, median 4 per participant). At each timepoint we measured markers of activation (HLA-DR+CD38+), cytotoxicity (CD107a+), cycling (Ki67+), exhaustion (TIGIT+PD-1+), 5' elongated HIV-2 RNA (median=71; P=0.04) and tended to be higher than Nef (median=71; P=0.04) and Poly A (4; P=0.063). HIV-2 transcripts. These trends persisted after normalization to the corresponding HIV-2 DNA regions. Ratios of S’ elongated/initiated HIV-2 RNAs (S’ elongation) were higher than Gag/initiated (P<0.04) and Read-Through/initiated (P<0.04). The ratios of S’ elongated/initiated (median=0.069) HIV-2 RNA also tended to be higher than Nef/initiated (distal elongation, 0.0022) and PolyA/initiated (completion, 0.0015; P=0.063 for both). We calculated that a median of 86% of HIV-2 transcripts are blocked at elongation, while 99% of transcripts are blocked at completion.

Conclusion: Differences in the levels of HIV-2 transcripts and ratios suggest that HIV-2 expression is inhibited by blocks to elongation and completion of HIV-2 transcription. These mechanisms, which are also observed in HIV-1 infection, likely contribute to latent infection with both viruses. Future studies aimed at curing HIV-2 should focus on determining the cellular factors underlying these blocks to transcriptional elongation and completion.

472 Variable Persistence of Non-Suppressible Viremia on Antiretroviral Therapy


University of Pittsburgh, Pittsburgh, PA, USA, *Harvard Th Chan School of Public Health, Boston, MA, USA, *Harvard University, Cambridge, MA, USA, *Frontier Science & Technology Research Foundation, Inc, Amherst, NY, USA, *University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, *Massachusetts General Hospital, Boston, MA, USA

Background: Nonsuppressible viremia (NSV) on antiretroviral therapy (ART) has been shown to originate from infected T-cell clones rather than viral replication. The variation and longitudinal persistence of NSV have not been well defined. To address this knowledge gap, we enrolled a longitudinal cohort of persons with NSV on stable ART.

Methods: We enrolled individuals living with HIV into a longitudinal ACTG cohort study of NSV who met the following inclusion criteria: At least 2 HIV-1 RNA values ≥20 and ≤1500 copies/copy/ml within 24 months prior to entry, at least 1 documented HIV-1 RNA value ≥20 and ≤1500 copy/ml within 12 months prior to entry and on uninterrupted ART for at least 12 months. Persistence of NSV was categorized into three groups among those without ART changes or interruptions using RNAs at week 0 (entry), week 24, 48 (all 3 required): 1) all RNAs <40 c/ml, 2) mix of RNAs <40 and ≥40 c/ml, and 3) all RNAs ≥40 c/ml. Analyses of participant characteristics at study entry compared the 3 groups by age, sex, pre-ART plasma HIV-1 RNA (log10 c/ml), pre-ART and entry CD4+ T-cell count (cells/mm3), entry CD4+ T-cell count (cells/mm3), entry CD4+CD8 ratio, years on ART at entry, and the proportion of RNAs ≥40 c/ml during 24 months on ART prior to entry.

Results: 22 participants were analyzed: 1 (1%) female; median age 58 (range 24-75); median years on ART 9.5 (range 2.9-26.8). The reported pre-study duration of NSV ranged to 10+ years. At week 48, 8 of 22 (36%) still had quantifiable viremia (≥40 c/ml). Only 3 (14%) participants had RNA ≥40 c/ml at weeks 0, 24 and 48; 10 (45%) had RNA <40 c/ml at all three weeks and 9 (41%) had mix of <40 and ≥40 c/ml. The median pre-ART CD4 T-cell count for groups 1, 2 and 3 were 98, 121 and 164 cells/mm3, respectively. The only factor significantly associated with persistence of NSV ≥40 c/ml was the proportion of plasma HIV RNA measurements that were ≥40 c/ml during the 24 months on ART prior to entry (p=0.001, Kruskal-Wallis Test) (Figure).

Conclusion: NSV was dynamic and consistently ≥40 c/ml at 0-48 weeks in only 14% of participants. Persistence of NSV over 48 weeks was only associated with the proportion of RNAs ≥40 c/ml prior to entry. These results are consistent with variable persistence of clones producing virus with the minority being stable, and the majority declining in size and/or producing less virus.
Different Impact of Latency Reversal in Cells From People With HIV Viremia and With ART Suppression

Remi Fromentin, Amélie Pagliuzza, Hiroshi Takata, William Brantly, Benjamin Varco-Merthy, Alain A. Boyer, Lydia Trautmann, Nicolas Chomont

Centres de Recherche du CHUM, Montréal, Canada; Oregon Health and Sciences University, Portland, OR, USA; US Military HIV Research Program, Silver Spring, MD, USA

Background: Induction of viral transcription using latency reversing agents (LRAs) is a promising approach to reduce the HIV reservoir but has shown modest effects in virally suppressed people with HIV (PWHA) on ART. The administration of LRAs at the time of ART initiation may enhance the efficacy of this approach, since latently infected cells may be more sensitive to latency reversal in a virologic setting. However, the effect of LRAs on infected cells isolated from untreated PWHA is largely unknown. Here, we compared the effect of the ingenol-based PKC agonist GS44495 alone or in combination with the HDACi romidepsin (RMD) on HIV transcription in cells from virologic and virally suppressed individuals.

Methods: We measured the effect GS44495A +/- RMD on HIV transcription in CD4+ cells isolated from the blood of 10 PWHA on ART with undetectable plasma viral load and in 18 untreated individuals (median = 3.51 ± 1.02 HIV RNA copies/mL). CD4+ T cells were conditioned or not for 4h with RMD (40nM) and pulsed for 30min with GS44495A (50nM) in the presence of antiretroviral drugs. HIV transcription was assessed 18h post-stimulation by RT-qPCR for cell-associated LTR-gag RNA.

Results: As expected, the addition of RMD enhanced the GS44495A-mediated induction of HIV transcription in cells from ART-suppressed PWH (mean fold change over GS44495A alone 35.4; p = 0.06). In cells from virocipientic participants, stimulation with GS44495A also led to a robust induction of HIV transcription (mean fold change 4.1, p<0.0001). However, the addition of RMD inhibited the induction of HIV transcription mediated by GS44495A (mean fold change 0.62, p = 0.0003). To determine whether this apparent decrease could be attributed to the death of infected cells upon latency reversal, we used the pan-caspase inhibitor Q-α-V-OPh 20µM. Inhibition of apoptosis not only rescued but also enhanced the levels of HIV transcripts measured in cells from virologic participants (mean fold increase in HIV RNA 2.1).

Conclusion: The combination of GS44495A and RMD leads to a marked reduction in the levels of HIV transcripts in cells isolated from virologic PWH. This decrease is abrogated by a caspase inhibitor, suggesting that latency reversal in a virologic setting leads to the death of the infected cells. Our results suggest that the administration of LRAs at the time of ART initiation, when the bulk of the reservoir is established, may reduce the frequency of persistently infected cells.

Strong Latency Reversal by C. megaloblytys Extract in ART-Suppressed PBMC and Humanized Mice

Khumoekei Richard, The Yuan, Riza Kothu, Emery T. Register, Paridhihama Sharma, Brian H. Ross, Pau Zuck; Caryn Cheneys, Jessemarioe Morris, Guoxin Wi, Karam Maunze, Kerstin Andrae-Marobela, Ian Tielje, Lois J. Montaner, \n
Wistar Institute, Philadelphia, PA, USA; Merck & Co, Inc, Kenilworth, NJ, USA; Phildophia FIGHT Philadelphia, PA, USA; University of Botswana, Gaborone, Botswana

Background: Latency reversing agents (LRAs) have had limited success in vivo, indicating a need for more potent agents. "Mukungulu," an extract prepared from the bark of Croton megalobotrys and traditionally used for HIV/AIDS management in Northern Botswana (Africa), is an LRA in latent HIV cell lines and contains protein kinase C-activating phorbol esters (PMID: 28970153). However, the properties of Mukungulu ex vivo in ART suppressed cells from PLWH and/or in vivo are not known.

Methods: Using cells from 12 PLWH suppressed on ART for > 3 years cell pellets and culture supernatants from 20 million PBMC in triplicate independent tests were assessed for p24 protein by Simoa (PMID: 33790087) after 72 hours treatment with Mukungulu (1 µg/mL) or anti-CD3/CD28 positive control. Isolated CD4 T-cells (5 million) were also tested in 3 donors. Intact and defective proviral DNA were assessed by IPDA (PMID: 30709139). 12 BTL-Humanized male mice were infected with HIVSU1A, suppressed with ART for 7 weeks (PMID: 36460646) and injected i.p. with 5 µg/mL Mukungulu (N=7) or PBS vehicle (N=5) and measured after 24 hours for pVL and vRNA from human cells. Analysis was done with Prism software.

Results: In PBMC from 12 ART suppressed donors, Mukungulu induced 0.30 ± 0.15 and 0.46 ± 0.24 pg/mL of p24 protein in pellets and supernatants, respectively, without cytotoxicity, compared to only 0.11 ± 0.06 and 0.17 ± 0.08 pg/mL of p24 induced by anti-CD3/CD28 (p = 0.06 and 0.07). In contrast, Mukungulu induced no detectable p24 in pellets or supernatants in isolated CD4+ cells, compared to anti-CD3/CD28 inducing 0.49 ± 0.12 and 0.38 ± 0.14 pg/mL of p24. Notably, p24 levels from PBMC pellets and supernatants induced by Mukungulu correlated well with intact provirus reservoir size (r² = 0.60 and 0.74, respectively) but not with defective provirus reservoir size (r² = 0.03 and 0.08). Finally, in humanized mice, Mukungulu induced 1336 ± 402 vRNA copies / million human cells plus a pVL of 391 ± 394 copies / mL, compared to no change by PBS, without obvious toxicities.

Conclusion: Mukungulu is a potent LRA ex vivo and in vivo. It induces virus production from the intact viral reservoir of CD4+ cells with ~2.5 more activity than anti-CD3/CD28. However, this reaction is dependent on PBMC and not isolated CD4+ cells, indicating that additional cell-types contribute to its mechanism of latency reversal.

Transcriptional HIV Reservoir Profiling Reveals a Role for Mitochondrial Functionality in HIV Latency

Shirley Man, Stefanie Kroeze, Jade Jansen, Teunis B. Geijtenbeek, Neeltje Kootstra

Academic Medical Center, Amsterdam, Netherlands

Background: Improved characterization of the HIV reservoir is crucial for devising effective cure strategies. We developed a strategy for isolating and characterizing the viral reservoir in peripheral blood from people with HIV (PWHA). We hypothesize that short abortive HIV transcripts are present in latently infected cells, and on this, we developed an innovative flow cytometry-/fluorescent in situ hybridization (flow-FISH) allowing HIV reservoir detection and cell sorting without prior activation. This method was used for direct ex vivo detection and isolation of HIV+ CD4 T cells harboring transcriptionally latent or active HIV and allowed for transcriptional analysis of the viral reservoir.

Methods: Peripheral blood mononuclear cells (PBMCs) from 15 ART-naive patients from the Amsterdam Cohort Studies were used for this study. Flow-FISH was performed with probes targeting either abortive (TAR+ Gag+) or elongated HIV transcripts (TAR+ Gag+), representing latently and productively infected cells, respectively. Flow cytometry sorting was used to isolate three distinct cell populations (i.e. TAR+ Gag+, TAR+ Gag+, and probe-negative) from CD4 T cells of five PWHA. The transcriptional profile was determined by 3’ RNA sequencing (RNAseq).

Results: Our flow-FISH method detected between 10-751 HIV+ cells per 10⁶ CD4 T cells in PBMCs from PWHA of which 1-47 per 10⁶ CD4 T cells harbored transcriptionally latent HIV (TAR+ Gag+) and 7-72 per 10⁶ CD4 T cells harbored transcriptionally active HIV (TAR+ Gag+; Figure A). Supervised RNAseq analysis allowed for the identification of transcriptionally significant genes that separate the isolated populations independently of person-related characteristics. Notably, we identified several differentially expressed mitochondrial genes in latent HIV (TAR+ Gag+) compared to productively infected (TAR+ Gag+) CD4 T cells (Figure B). Interestingly, enhancing mitochondrial function increased HIV transcriptional activity in latently infected CD4 T cells from PWHA.

Conclusion: Our flow-FISH approach was able to detect and differentiate between cells with transcriptionally latent or active HIV in PBMCs from PWHA without the need for ex vivo stimulation. Transcriptional profiling showed an association between diminished mitochondrial functioning and the transcriptional activity of the viral reservoir. These findings underline the relevance of altered cellular metabolism in HIV infection, and support the development of therapeutics that take this into consideration.

Table 1. HIV-1 p24 Induction by C. megaloblytys Extract

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<tr>
<td>RMD</td>
<td>0.60 ± 0.06</td>
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477 Investigating the Effects of Chromatin Spatial Folding on HIV-1 Latency
Nawal K. Al Burtanami, Jose De Las Heras, Khushi Goel, Alex Ward, Helen Rowe, Ariberto Fassati
1University College London, London, United Kingdom, 2University of Edinburgh, Edinburgh, United Kingdom
3Queen Mary University of London, London, United Kingdom

Background: Persistence of HIV-1 in latent reservoirs is a major obstacle that prevents its eradication and cure. Understanding the mechanisms governing HIV-1 latency is crucial to eliminate this reservoir. Spatial folding of chromatin governs cellular gene expression. However, how 3D genome organization affects HIV-1 latency is unknown.

Methods: To investigate the impact of chromatin spatial folding on HIV-1 latency, we used a comparative approach that takes advantage of the different integration profiles of wild type (WT) HIV-1 and a capsid mutant virus N74D. We mapped unique integration sites (UISs) for HIV-1 WT and N74D to DamID and HiC data. Next, we investigated how different integration site distribution may affect latency. Jurkat cells were transduced with either WT or N74D single cycle HIV-1 vectors expressing GFP from the LTR. GFP+ cells were sorted after 48 hr, and latency was established. Lastly, we identified UISs functionally relevant for latency, located in infected cells (WT and N74D) were stimulated serially by TCR engagement followed by inhibition of HDAC6 and sorted into reversible (GFP-) and deep (GFP-) latency populations at each step.

Results: We observed that WT HIV-1 integrated more frequently in A1 compartment, whereas N74D virus integrated more frequently into lamina associated domains (LADs). These results were confirmed in ART-treated patients’ cells. We found that WT virus becomes latent more slowly and can be reactivated more efficiently than N74D virus by TCR stimulation or PMA. Epigenetic profiling showed that the pan histone deacetylase inhibitor SAHA and the G9a inhibitor BIX-0124 reversed both WT and N74D latency with equal efficiency; however, selective inhibition of HDAC6, inhibition of FOXO-1 or A2aL reversed WT more potently than N74D latency. Integration site analysis on sorted GFP+ and GFP- cell populations revealed that, in WT HIV-1 infected cells, latency was associated with significantly fewer UISs in the A1 compartment and significantly more UISs in the B1 compartment. Deep latency was associated with more UISs in intergenic regions and fewer UISs in exonic regions than reversible latency. In N74D virus infected latent cells, deep latency was associated with greater distance of UISs from transcriptional start sites.

Conclusion: We propose that deep latency may be linked to integration into particular 3D chromatin regions enriched for certain epigenetic marks and gene clusters, which might be specifically targeted to either induce, or prevent, virus reactivation.

478 Modulation of IRF7-Driven Transcription as a Strategy to Control HIV-1 Latency
Ifeany Ezenwemueli, Edurne Garcia-Vidal, Eudaldi Felip, Bonaventura Clotet, Roger Badia, Estel Ballana, Eva Rivera-Muñoz
1University of California San Francisco, San Francisco, CA, USA, 2Icahn Institute for AIDS Research, Badalona, Spain

Background: The persistence of a latent viral reservoir represents a major barrier shared by current HIV cure strategies. Emerging evidence suggests that modulation of innate immunity could impact viral latency and contribute to the clearing of HIV reservoir. Our previous data indicated that IRF7 expression correlates with HIV latency reversal. Here, we demonstrate the key role of IRF7 in HIV-1 transcription and latency, identifying also novel therapeutic agents useful in HIV eradication.

Methods: Latency was evaluated by non-clonal models drug-treated, alone or in combination with known LRAs. qPCR and WB were used to assess gene and protein expression. IRF7 loss and gain-of-function models were developed by siRNA or plasmid overexpression. IRF7 role in HIV-1 transcription was evaluated by measuring LTR-driven transactivation in TZM-bl cells and co-immunoprecipitation. Immunopositive expression of ex vivo treated CD4+ T-cells from ART-suppressed HIV+ subjects was performed by flow cytometry and latency reactivation/promoting activity was measured by qPCR in cell supernatant.

Results: IRF7 expression correlates with latency reversal/promoting capacity of LRAs/LRAs, including PMMA, PMA, and the JAK2 inhibitors fedoratinib and pacitaxin among others (r2= 0.8, p-value=0.0012). Downregulation of IRF7 impaired the latency reversal capacity of LRAs (p=0.005). On the contrary, overexpression of IRF7 enhances Tat-mediated transactivation of integrated HIV in the presence of LRAs (at least 5% increase, p=0.05). Co-immunoprecipitation studies showed physiological interaction between Tat protein and IRF7, relating IRF7 to HIV-1 transcription control. The JAK2 inhibitor pacitaxin downregulated IRF7 expression and thus, was used to further explore IRF7 role in HIV latency. Pacitaxin significantly blocked HIV-1 reactivation both alone or in combination with PMA or VOR in non-clonal models of HIV-1 latency (50% reduction, p=0.05) and ex vivo in CD4+ T cells from ART-suppressed HIV+ subjects (10% reduction). Immunofluorescence characterization of pacitaxin-treated primary CD4+ T cells from FLWH showed no major changes on CD4+ T cell subsets nor activation markers. Moreover, pacitaxin significantly reduced the presence of multiplicated HIV transcripts in primary CD4+ T cells.

Conclusion: IRF7 controls latent HIV-1 transcription and plays a role in both HIV-1 reactivation or blockade. Moreover, modulation of IRF7 expression through pharmacological agents might represent an asset to control HIV-1 cure strategies.

479 Reversing HIV-1 Latency by Targeting RasGRP1-Dependent Biogenesis of P-TEFb
Uri Mbonye, Ana Bellomo, Eleonora Elahem, Lucia G. Donadio, Maria J. Comin
1Case Western Reserve University, Cleveland, OH, USA, 2National Institute of Industrial Technology, Buenos Aires, Argentina

Background: The deliberate reactivation of latent HIV-1 to enable clearance of persistently infected memory T cells – the Shock and Kill strategy – has limited success because efficient, non-toxic latency-reversing agents (LRAs) remain to be discovered. We are taking a direct approach to LRA development based on the principle that proviral reactivation is tightly coupled to the posttranscriptional biogenesis of P-TEFb, a cellular transcription elongation factor whose expression is highly restricted in resting memory T cells. Recently, we reported that naturally occurring diacylglycerol (DAG)-mimicking protein kinase C (PKC) agonists stimulate the posttranscriptional expression of the cyclin T1 (CyclT1) subunit of P-TEFb to reactivates latent HIV-1 primarily via the RasGRP1-Ras-Raf-MEK-MAPK ERK1/2 signaling pathway rather than through PKC enzymes. Here we described synthetic agonists that preferentially bind RasGRP1 over PKC and can safely activate P-TEFb to reverse HIV-1 latency in primary CD4+ T cells.

Methods: Synthetic DAG indololactones, with known differential affinities for PKC and RasGRP1, were tested for their ability to stimulate P-TEFb expression in healthy donor-derived memory CD4+ T cells. Combinatorial LRA studies were performed in a primary CD4+ T-cell latency model to examine the effectiveness of DAG indololactones at synergizing with LRAs that target proviral transcription initiation.

Results: Three of the 4 DAG indololactones we tested were more effective at inducing CyclT1 and active P-TEFb than the T-cell activation markers CD69 and CD25. The DAG indololactone 2A-127, which preferentially binds RasGRP1 over PKC more than 60-fold, synergized with the HDACi SAHA in reactivating latent HIV-1 in primary T cells. DAG indololactones and other DAG-mimicking agonists stimulate CyclT1 protein synthesis by activating mTORC1 kinase through ERK1/2 MAPK signaling. DAG indololactones can stimulate the posttranscriptional expression of P-TEFb in memory T cells at concentrations below the threshold needed to induce T-cell activation through a RasGRP1-mediated ERK1/2-mTORC1-S6K-rpS6 pathway. These agents can synergize with HDAC inhibitors, thereby bolstering the hypothesis that a two-pronged strategy that targets P-TEFb biogenesis and stimulates RNA polymerase II recruitment to the HIV-1 promoter is needed to reverse HIV-1 latency efficiently.
480 Monovalent and Bivalent SMAC Mimetics Reverse HIV Latency and Decreases the HIV Reservoir

Youry Kim, Kiho Tanaka, Jesslyn Ong, Carolin Tumpach, James H. McMahon, Ajantha Rhodes, Rebecca Hoi, Steven G. Deeks, Sushama Telwatt, Michael Roche, Sharon R. Lewin

*Peter Doherty Institute, Melbourne, Australia, 1Alfred Hospital, Melbourne, Australia, University of California San Francisco, San Francisco, CA, USA*

**Background:** Latently infected CD4+ T cells persist in people living with HIV (PLWH) despite suppressive antiretroviral therapy (ART). This persistence may be due to the over-expression of pro-survival proteins such as the inhibitors of apoptosis (IAP) proteins. SMAC mimetics (SMACm) are small molecule compounds that inhibit IAPs, leading to their degradation and ultimately the activation of apoptosis. While bivalent SMACm are known to be more potent than monovalent SMACm, they cause significant toxicities, which have not been seen in clinical trials of monovalent SMACm. Here we investigated whether monovalent and bivalent SMACm could reverse latency and/or deplete the reservoir.

**Methods:** Latency reversal by monovalent (GDC0197, GDC0152, LCL161, Kevimabant) and bivalent (AZD5882, BIV6) SMACm was assessed in J-Lat 10.6 cells (flow cytometry for GFP expression); the dual-reporter primary CD4+ T cell latency model Morpheus (flow cytometry for productive marker mCherry); and ex vivo CD4+ T cells from PLWH on ART (using HIV transcriptional profiling by digital PCR). Depletion of infected cells in ex vivo cultures was measured by the Intact Proviral DNA assay. Proliferation and function of HIV-specific CD8+ T cells following treatment with SMACm was measured using HIV-specific tetramers and flow cytometry.

**Results:** The bivalent SMACm AZD5882 (100nM) was the most potent latency reversal agent in J-Lat 10.6 cells (fold change (FC) reactivation over untreated=22.92, p=0.03) and in the primary cell latency model (FC=1.43, p=0.03). AZD5882 partially overcame all blocks in HIV RNA transcription inducing multiply spliced HIV transcripts (FC over untreated=2.35, p=0.03). AZD5882 treatment also led to a decline in intact HIV provirus (FC=0.51, p=0.02). The monovalent SMACm GDC0197 reversed latency in cell lines (FC=1.62) and primary T cells (FC=1.30, p=0.01); but could only induce HIV RNA transcription initiation (FC=4.26, p=0.03), and not elongation or completion. GDC0197 induced the proliferation of tetramer positive cells (FC=5.63 over untreated, p=0.0001) and enhanced killing of Gag peptide-loaded target cells (FC=1.47 killing over untreated). This was not observed with AZD5882.

**Conclusion:** Bivalent SMACm can reactivate latent HIV and deplete the reservoir. Monovalent SMACm although less potent in latency reversal, may have a novel role in enhancing clearance of the reservoir through altering antigen presentation and inducing greater CD8+ T cell mediated killing.

481 Optimal Administration Timing of Latency Reversal Agents to Reduce Effectively the HIV Reservoir

Erick De La Torre Tarazona, Sergio Serrano-Villar, Raúl Vaquero, Marta Raya, Laura Lina García, Soledades Sánchez-Palomino, Teresa Aldímez-Echevarría, Rafael Micán, Adriá Curran, Melchor Briesa, José Alcamí, Inma Jarrín, Santiago Morente

*Hospital Ramón y Cajal, Madrid, Spain, 1Institute of Health Carlos III, Madrid, Spain, 2Hospital Clinic of Barcelona, Barcelona, Spain, 3University Hospital Gregorio Marañón, Madrid, Spain, 4La Paz University Hospital, Madrid, Spain, 5Hospital Universitario de la Vall d’Hebron, Barcelona, Spain, 6Hospital Universitario de San Espíritu, Palma de Mallorca, Spain, 7Instituto de Salud Carlos III, Majadahonda, Spain*

**Background:** The administration timing of Latency Reversal Agents (LRA) may impact the success of strategies aimed to HIV functional cure. Maraviroc (MVC) is an antiretroviral drug that exhibits HIV latency-reversing properties by activating viral transcription through NF-κB pathway. We aimed to evaluate the efficacy on HIV-DNA reduction following MVC administration at the time of ART initiation (with detectable viral load), or in patients under suppressive ART.

**Methods:** We compared people with HIV initiating ART with a regimen including MVC (Cases, n=12) to two different control groups: Control 1 (n=26), who initiated ART not containing MVC; and Control 2 (n=21), who had undetectable HIV-RNA on ART and switched to a MVC-containing regimen. Cases and Control 1 groups were matched by age, sex, and the number and type of drugs in ART regimen (excluding MVC). We determined the HIV reservoir size by measuring integrated HIV-DNA in stored PBMCs. We estimated the HIV reservoir variation with multivariate linear regression models adjusted for age, baseline CD4 counts, baseline HIV-RNA and baseline HIV-DNA.

**Results:** Pre-ART, median CD4 counts (cells/mm³) were 96 (IQR: 36.5-204.0) in Cases and 351.5 (IQR: 237.0-494.0) in Control 1, and 456.0 (IQR: 264.5-598.5) prior MVC inclusion in Control 2. Pre-ART, the HIV reservoir size (median copies of integrated HIV/million cells) was 2368.0 (IQR: 38.6-9101.0) in Cases and 1583.0 (IQR: 97.3-5270.0) in Control 1. After a median of 79.7 weeks (IQR: 72.0-116.0) from ART initiation, the HIV reservoir size decreased significantly in both groups (97.5 [IQR: 5.4-552.9] in Cases and 720.5 [IQR: 72.6-2578.0] in Control 1, p<0.01). Control 2 group displayed an HIV reservoir size of 661.3 (IQR: 130.4-4499.0) and 416.0 (IQR: 31.2-965.3) before and after switching to a MVC-containing regimen, respectively (p<0.01). Multivariate analysis showed that Cases had a 12-fold and 11-fold greater HIV reservoir decline than Control 1 and Control 2, respectively (p<0.01).

**Conclusion:** Administering an LRA during ART initiation with detectable viremia more effectively reduces the HIV reservoir size than in patients with undetectable viral load. This finding can inform the design of clinical trials based on the shock and kill strategy.

482 The Fraction of HIV Reservoir Variants Neutralized by Autologous IgG Correlates With Time to Rebound

Mauro A. García, Junlin Zhao, Joseph Varriale, Anna Farrell-Sherman, Jun Lai, Anthony Abeyta-Lopez, Natalie F. McMyn, Rebecca Hoi, Michael J. Pelleo, Francesco R. Simonetti, Steven G. Deeks, Lillian Cohn, Robert F. Siliciano, Janet M. Siliciano

*The Johns Hopkins University, Baltimore, MD, USA, 2Fred Hutchinson Cancer Center, Seattle, WA, USA, 3University of California San Francisco, San Francisco, CA, USA*

**Background:** During untreated infection, HIV-1 rapidly evolves to escape contemporaneous, autologous neutralizing antibodies (anAbs). At the time of ART initiation, archived reservoir isolates may be sensitive or resistant to anAbs. Factors such as time to rebound, and the genotype of rebound virus are difficult to predict among analytically treatment interruption (ATI) cohorts. Elucidating the factors that govern these phenomena can inform cure strategies aiming for ART-free virologic control. We hypothesize that (1) anAb-resistant virus detected in the reservoir prior to an ATI may be identical, or genetically similar, to virus that rebounds post ART, and (2) higher percentages of anAb-sensitive reservoir variants may be associated with a longer time to rebound post ART.

**Methods:** Among 9 study participants who underwent an ATI without intervention, we measured inhibition of outgrowth by contemporaneous anAbs. At the time of ART initiation, archived reservoir isolates may be sensitive or resistant to anAbs. Factors such as time to rebound, and the genotype of rebound virus are difficult to predict among analytically treatment interruption (ATI) cohorts. Elucidating the factors that govern these phenomena can inform cure strategies aiming for ART-free virologic control. We hypothesize that (1) anAb-resistant virus detected in the reservoir prior to an ATI may be identical, or genetically similar, to virus that rebounds post ART, and (2) higher percentages of anAb-sensitive reservoir variants may be associated with a longer time to rebound post ART.

**Results:** We observed donor-to-donor variability in the ability to neutralize anAb-resistant virus, which may impact the design of clinical trials based on the shock and kill strategy.
selective ATI enrollment. Lastly, ATI studies using time to viral rebound as an outcome measurement should consider the anAb response when designing trials and interpreting results.

**483 Plasma Correlates of Rebound After Discontinuation of ART in Persons Living With HIV-1 (PLWH)**

Malika A. Boudries, Dan H. Barouch, Boris D. Juelg, Victoria E. Walker-Spellers
Beth Israel Deaconess Medical Center, Boston, MA, USA, "Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA

**Background:** The discovery of biomarkers that predict viral rebound after ATI would significantly contribute to the HIV cure field. Further, the discovery of biomarkers predicting viral rebound after ART discontinuation will inform and guide ATI studies to understand who is more likely to experience a viral rebound and could help select participants for ART interruption studies to make ART discontinuation safer and more effective.

**Methods:** We initiated a multi-center, open-label trial of three monthly intravenous (IV) administration of 20 mg/kg each of PG121, PGDM1400, and VRC07-523LS (n=12) in persons living with HIV-1 (PLWH) following discontinuation of antiretroviral therapy (ART). ART was interrupted 2 days after the first broadly neutralizing monoclonal antibodies (bnAb) infusion.

We collected plasma from all participants two days before ATI, multiple times off ART, and after rebound. We conducted high-throughput transcriptomics, proteomics, and metabolomics at all time points in all participants.

**Results:** We investigated protein signatures modulated after ATI, before detectable rebound (VL<200 cp/ml), and after rebound (VL>200 cp/ml) compared with baseline (pre-ATI). We observed a significant increase (FDR q value < 0.05) of T cells, immune activation, and proinflammatory signatures preceding detectable rebound (VL<200 cp/ml) that were augmented after rebound. Signatures of activated proinflammatory macrophage M1, response to interferon-alpha and gamma, as well as proinflammatory cytokines and chemokines (CXCL10, CXCL9, TNFRSF1B, CD14, CSF1) were elevated before detectable rebound compared with pre-ATI.

Metabolic pathways showed a value < 0.05) of T cells, immune activation, and proinflammatory signatures following rebound virus (Fig 1). We found no evidence of viral reactivation (VL>200cp/ml), and after rebound. Metabolic pathways showed significant upregulation of the pro-survival factor BIRC5 and low levels of the integrin component CD29.

**Conclusion:** Our preliminary observations highlight the role of proinflammatory signatures and macrophages as potential plasma biomarkers to predict imminent rebound following ATI.

484 Common T-Cell Features Predicting Time to Rebound in Interventional and Non-Interventional Treatment

Tonggui Ma, Ashley F. George, Reuben Thomas, Min-Gyoung Shin, Mauricio Montano, Satish R. Pillai, Katherine S. Pollard, Rajesh T. Gandhi, Jonathan Z. Lii, Davey M. Smith, Steven G. Deeks, Ole S. Søgaard, Ole S. Søgaard, Nadia R. Roan, 1Gladstone Institutes, San Francisco, CA, USA, 2Wadsworth Research Institute, San Francisco, CA, USA, 3Massachusetts General Hospital, Boston, MA, USA, 4 Brigham and Women’s Hospital, Boston, MA, USA, University of California San Diego, La Jolla, CA, USA, 5University of California San Francisco, San Francisco, USA, 6Aarhus University Hospital, Aarhus, Denmark

**Background:** Immunological features predicting time-to-rebound during analytical treatment interruption (ATI) may differ between cohorts, and be impacted by whether or not therapeutic interventions were used. Here, we used CyTOF to identify shared and unique pre-ATI features of T cells associated with time-to-rebound in the non-interventional ACTG A5343 cohort and 3 interventional Danish cohorts.

**Methods:** We used CyTOF to identify shared and unique pre-ATI features of T cells associated with time-to-rebound in the non-interventional ACTG A5343 cohort and 3 interventional Danish cohorts.

**Results:** Using cluster-resolution optimization, nine and five clusters were identified from the A5343 and the 3 collective Danish cohorts, respectively. Cluster 9 from A5343 and Cluster 3 from TEACH were both significantly (>0.01) positively associated with longer time-to-rebound. Cluster 9 consisted of CD4+ and CD8+ T cells, while Cluster 3 consisted of CD8+ T cells expressing high levels of the T resident memory (Trm) marker CD103, and high levels of the integrin component CD29. By contrast, Cluster 9 expressed low levels of BIRC5 and high levels of the co-stimulatory molecule CD28. Cluster 3 from the Danish cohorts consisted of CD8+ T cells expressing high levels of BIRC5 and low levels of CD29. These cells also expressed high levels of CXCR4 and CCR7, suggesting homing to inflamed and lymphoid tissues. Our observation that CD8+ T cells from both A5343 and Cluster 9a and Danish Cluster 3 expressed high levels of BIRC5 and low levels of CD29 suggests that phenotypic features predicting longer time-to-rebound can be shared among non-interventional and interventional ATI cohorts.

**Conclusion:** CD8+ T cells expressing high levels of BIRC5 and low levels of CD29 predict longer time-to-rebound in both interventional and non-interventional ATI cohorts. These “pro-survival” CD8+ T cells may be better able to survive during the early post-ART period and thereby slow down viral rebound upon ATI.

485 Dynamics of the Intact HIV Reservoir During ART Following Analytical Treatment Interruption

Maegan R. Manning, Jana Blazkova, Jesse S. Justement, Victoria Shi, Brooke D. Kennedy, M. A. Rai, Catherine A. Seamon, Kathleen R. Gittens, Michael C. Sneller, Susan Moir, Tae-Wook Chun

**National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

**Background:** To assess efficacy, clinical trials evaluating therapeutic agents aimed at achieving HIV cure or sustained virologic remission require analytical treatment interruption (ATI). It has been shown that ATI results in the expansion of HIV reservoirs in people living with HIV (PLWH) with rebounding plasma viremia; however, the dynamics of the intact HIV reservoir during ART in PLWH who underwent ATI has not been fully delineated. We conducted longitudinal measurements of intact HIV proviral DNA, residual plasma viremia, and biomarkers before and during ATI and analyzed reinitiation of ART in PLWH who participated in previous ATI trials.

**Methods:** We studied two cohorts of PLWH who initiated ART during the acute/early (A/E, n=25) or the chronic (Chronic, n=19) phase of infection. We determined the levels of intact HIV proviral DNA, residual plasma viremia, and biomarkers before and during ATI, and 24 and 52 weeks following the re-initiation of ART. Levels of intact HIV DNA were determined using the intact proviral DNA assay (IPDA). Residual plasma viremia was measured using an automated instrument. Levels of plasma biomarkers were measured by an automated ELISA.
Results: Both groups experienced a significant increase in the level of intact HIV viral DNA following ART. After the re-initiation of ART (Post-ATI), the intact HIV DNA burden in the A/E group normalized to the baseline level (P=0.1245) by Week 24. However, in the Chronic group, the level of intact HIV DNA remained elevated at Post-ATI Week 24 and 52 (P=0.0069 and P=0.0042, respectively) compared to that of baseline. There was no difference in the residual plasma viremia between the baseline and Post-ATI Week 24 in the A/E group (P=0.1556). However, in the Chronic group, the level of residual plasma viremia was significantly higher at Post-ATI Week 24 compared to baseline (P=0.0181). There were significant increases in the levels of soluble PD-1 (P=0.0066) and perforin (P=0.0094) from baseline to Post-ATI Week 24 in the Chronic but not in the A/E group.

Conclusion: We conclude that ATI differentially affects the dynamics of the HIV reservoir, residual plasma viremia, and certain biomarkers following the re-initiation of ART in PLWH who initiated ART during the acute/early versus chronic phase of infection. Thus, delineating the underlying mechanisms by which the A/E group achieved faster normalization compared to the Chronic group of immunologic and virologic markers could be informative for the development of future therapies.

486 Immune Responses and HIV Reservoir Evolution From Pre-ART to 5 Years Into Post-Treatment Control

Leah Carrere1, Miriam Rosas-Umbert1, Benjamin Bone1, Giacomo S. Frattaroli2, Isabella Roxeto3, Xiaodong Lian1, Martin Tolstrup1, Ce Gao1, Mariane H. Schleimann1, Xu G. Yu1, Jesper D. Gun1, Mathias Lichterfeld1, Ole S. Søgaard2, 1Rajan Institute of MGT, MFT and Harvard, Cambridge, MA, USA, 2Aarhus University, Aarhus, Denmark

Background: In the randomized, controlled eCLEAR study focusing on the administration of the broadly neutralizing antibody 3BNC117 at the time of ART initiation, one post-treatment controller was identified. He initiated ART and 3BNC117 during primary HIV-1 infection (plasma viral load of 188,945 copies/mL and CD4 counts of 470 cells/mm³) and has had sustained virologic control for 5.3 years following ART interruption (ATI). However, immunological and virologic mechanisms in this post-treatment controller are poorly understood.

Methods: Viral reservoir cells were evaluated using quantitative in vitro viral outgrowth assays, duplex ddPCR (3dPCR), near full-genome proviral sequencing (FLIP-Seq) and matched integration site and proviral sequencing (MIP-Seq). Single cell transcriptome, surface protein and T-cell receptor profiling of 14,230 cells was performed with the 10x platform. HIV-1-specific CD8 T cells were analyzed using the activation-induced marker (AIM) assay.

Results: The person did not have any known protective HLA alleles and plasma samples repeatedly tested negative for antiretroviral drugs. Replication-competent proviruses were detected during ATI at a frequency of 0.2-0.5 infectious units/million CD4 T cells. Intact proviruses/million CD4 T cells declined during ART. PreART intact proviruses were predominantly (85%) located in ordinary genic locations, although two clones of intact proviruses in ZNF genes on chromosome 19 were observed. During the ATI stage, two large clones of intact proviruses were observed, one integrated in the centromere region of chromosome 4, and one in the peri centromere region of chromosome Y; one single provirus integrated in the centromere region of chromosome 9 was also noted. A strong HIV-1-specific CD8 T response towards Gag was maintained during the ATI, whereas responses towards Pol, Nef and Env decreased. Single cell analysis showed widespread CD4 T cell activation during ATI compared to pre-ATI and upregulation of cytotoxic and antiviral markers (GZMA, CX3CR1, APOBEC3G) by a subset of highly clonally expanded Th1 CD4 T cells.

Conclusion: Post-treatment control in this individual was associated with persistence of genome-intact HIV-1 proviruses that grew out under in vitro tissue culture assays but not under physiological in vivo conditions. This is possibly due to a strong immune-mediated selection of intact proviruses in heterochromatin locations that may have a weaker ability to drive rebound in vivo.

487 Intact HIV Reservoir Levels Are Stable After Short-Term ART Interruption

Prerana Shrestha1, Autumn Kittelson2, Meghan Melberg3, Evgenia Aga4, Ronald J. Boschi5, Jintanat Ananworanich5, Robert Coombs6, John W. Mellors7, Alan Landay7, Bernard J. Macatangay6, Steven G. Deeks8, Rajesh T. Gandhi9, Davey M. Smith8, Jonathan Z. Li1, for the AIDS Clinical Trials Group A5345 Study Team

1Brigham and Women’s Hospital, Cambridge, MA, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3Thai Red Cross AIDS Research Centre, Bangkok, Thailand, 4University of Washington, Seattle, WA, USA, 5University of Pittsburgh, Pittsburgh, PA, USA, 6Rush University Medical Center, Chicago, IL, USA, 7University of California San Francisco, San Francisco, CA, USA, 8Massachusetts General Hospital, Boston, MA, USA, 9University of California San Diego, San Diego, CA, USA

Background: As HIV cure remains a high priority for HIV research, analytical treatment interruption (ATI) in clinical studies remain vital to understand mechanisms for ART-free HIV remission. However, the impact of short-term treatment interruption on the intact HIV reservoir remains unclear.

Methods: We evaluated participants who underwent treatment interruption as part of the ACTG A5345 trial. Participants had been on suppressive ART ≥2 years and comprised two groups: individuals who initiated ART during chronic infection (n=33) and during early infection (n=12). HIV reservoir levels were measured at three time points: pre-ATI, during the ATI and — 24 weeks of viral re-suppression on ART (Step 3). We quantified levels of unspliced cell-associated RNA (CA-RNA), intact, defective, and total proviruses by the intact proviral DNA assay (IPDA). Residual viremia was measured by the integrase single-copy assay (iSCA). Wilcoxon rank-sum test was used for between-group comparison and Wilcoxon signed-ranks test for within-person comparison.

Results: The median duration of ATI was 4 weeks in the study. Compared to the pre-ATI time point, levels of intact, defective, and total HIV DNA levels were significantly increased during the ATI for both the early and chronic-treated groups. Approximately 24 weeks after viral re-suppression on ART, almost all levels of proviral measures (including intact and total HIV DNA) had returned to their baseline levels with no significant differences compared to the pre-ATI time point for both early and chronic-treated participants (Figure). Compared to pre-ATI, there was also no significant increase in CA-RNA levels 24 weeks after viral re-suppression on ART. At all time points, levels of CA-RNA, intact, defective, and total HIV DNA levels were higher in chronic-treated compared to early-treated individuals. Early after ATI (median 1 week), higher levels of residual viremia by the iSCA was significantly associated with a shorter time to viral rebound ≥1000 HIV-1 RNA copies/mL amongst all participants (Spearman r = -0.60, p<0.01).

Conclusion: Short-term ATI does not irreversibly change the reservoir size as reflected by stable levels of CA-RNA, or intact and total HIV DNA after viral re-suppression. High-level viral rebound can be predicted by early signals of very low viremia.

488 Clonal Expansion and Driving Forces of HIV-1 Persistence in Anatomic Compartments

Annemarie Glassey1, Wenjie Wang2, Lindsey Adams3, Mary-Eлизabeth Zipparo2, Robert Gorelick2, Stephen Hewitt2, Sharija Rajan4, Kathryn Lurain5, Ramya Ramaswami5, Chuen-Yen Lau6, Xiaodong Lian6, Julia Patrow2, Thuy Nguyen2, Frank Malardelli6, 1National Cancer Institute, Frederick, MD, USA, 2Leidos Biomedical Research, Inc, Frederick, MD, USA, 3National Institutes of Health, Bethesda, MD, USA, 4University of California San Francisco, San Francisco, CA, USA, 5University of Pittsburgh, Pittsburgh, PA, USA, 6National Cancer Institute, Bethesda, MD, USA

Background: Upon infection, HIV quickly disseminates and establishes tissue-specific infection throughout the body. The persistence mechanisms of HIV in tissues are complex, with viral and local environment interaction contributing to tissue-specific pathogenesis. To investigate the distribution of HIV populations in anatomic compartments, we characterized HIV-infected populations in tissues obtained at autopsy from individuals on suppressive therapy.
**Methods:** HIV-DNA in tissues was quantified by single copy and multiplexed LTR/gag digital droplet PCR. Single genome sequences of proviral gag or pol were assessed by average pairwise distance (APD) (genetic diversity) and Slatkina-Maddison analyses. Proviral integration sites (IS) were obtained to assess diversity and clonal expansion rate/patterns; alpha (Simpson) and beta diversity (Bray-Curtis) indices were calculated to quantify intra- and inter-tissue diversity of clones.

**Results:** Eight donors (median age = 50 y) expired from comorbid illnesses (5 neoplasms, 1 cardiac disease, 2 infection) underwent autopsy within 3-48 hours. HIV-DNA was present in all tissues with highest concentrations in lymph node (43-720 copies/µl cells), and lowest in brain (1-9 copies). HIV-LTR/gag quantification revealed diverse proviral structures with variable proportions of gag-deleted proviruses (0.1%-87.4%). Across tissues, proviruses harbored variable levels of hypermutations (6.2-44.4%) but were not compartmentalized (APD = 0.2%-0.9%) and intermingled if hypermutated sequences were discarded. We obtained 914 IS from 3 donors with median (range) of 26 (3-163) IS per tissue. In 2/3 donors, clonal expansion rates were significantly different but not tissue-specific with 0-91.9% of clonal proviruses per tissue. Median (range) intra- and inter-tissue diversity indexes were 0.95 (0.53-0.1) and 0.93 (0.64-1) suggesting non-diverse but distinct proviral populations across tissues. In one donor with >15 tissues analyzed for IS, we observed significant difference in intra-tissue diversity between neoplastic and non-neoplastic (p = 0.03) and in inter-tissue diversity between lymphoid vs non-lymphoid tissues (p = 0.002).

**Conclusion:** During therapy, HIV-infected cells are widely distributed in tissues but subject to differential pressures allowing the selection of proviruses with variable levels of defects and hypermutations. Clonal expansion significantly contributes to the proviral landscape. Our data suggests the role of local immune responses in shaping the anatomic proviral landscape.

**Impact of BACH2 on the Formation of HIV Latent Reservoirs in Humanized Mouse Model**

**Hongbo Gao, Liang Shan**

**Washington University in St Louis, St Louis, MO, USA**

**Background:** A pivotal challenge in achieving a cure for HIV is the limited understanding of the mechanisms governing the formation of latent reservoirs. Intriguingly, HIV integration site analysis studies have revealed unusually frequent integrations into the BACH2 gene. More importantly, inserted proviruses are nearly exclusively located upstream of the BACH2 start codon and share the same transcriptional orientation as the host gene, which leads to an increase in BACH2 transcription. The recurrent HIV-1 integration at the BACH2 locus and upregulation of the host gene expression suggest that BACH2 plays an important role in HIV reservoir seeding and maintenance.

**Methods:** To investigate the impact of BACH2 on the HIV latent reservoir in vivo, humanized mice engrafted with BACH2 knockout (BACH2-KO) or control CD4+ cells were generated. Engrafted mice were infected with HIV followed by the administration of ART for six weeks. Subsequently, the frequency of tissue HIV DNA was assessed, and a portion of the splenic cells were transferred to immunodeficient mice for viral rebound analysis. In addition, we evaluated the influence of BACH2 on the conversion of CCRC5+ CD4+ T cells into memory cells.

**Results:** As expected, we observed a notable reduction in total B cells in mice reconstituted with a BACH2-KO immune system in comparison to the control animals. Concurrently, BACH2-KO group had a slight decline in the number of naïve T cells. Notwithstanding, BACH2 deficiency did not affect the quantities of CD4+ central and effector memory cells. In HIV-infected mice, the viral loads were comparable between the BACH2-KO and control groups. In mice on ART, a lower frequency of HIV DNA was observed in the BACH2-KO group. In splenic cell transfer experiments, a 100% rebound was detected in the control mice (5/5), contrasted by a 50% rebound (4/8) in the BACH2-KO group. Mechanistically, we found that BACH2-KO CCRC5+ cells were unable to convert into long-lived central memory cells.

**Conclusion:** Our study shows that BACH2 is required for the conversion of CCRC5+ cells to central memory cells, and the formation of HIV reservoirs in long-lived central memory cells was abrogated in the absence of BACH2. Our study provides insights into the mechanisms underlying the establishment and stability of HIV latent reservoirs and identifies avenues for targeted interventions.

**Tissue-Resident TCR Repertoires Linked to HIV Persistence**

**Antoine Chaillou,1 Alan Wells,2 Ravi Goyal,3 Nadia R. Roant,4 Angela Jones,5 Karen Beer,1 Simon Mallal,1 Celestine Wanjalla,1 Magali Porrocha,1 Geonna Caballeria,5 Pinyi Du,6 Caroline Ignacio,1 Davey M. Smith,1 Sara Gianella1**

1University of California San Diego, La Jolla, CA, USA, 2University of California San Francisco, San Francisco, CA, USA, 3Vanderbilt University, Nashville, TN, USA

**Background:** Clonal expansion of infected CD4+ T cells is a mechanism of HIV persistence, driven by homeostatic, integration site-driven, and antigen-driven proliferation. We profiled the composition and diversity of TCR repertoires across 18 tissues and blood of 19 people with HIV (PWH) from the Last Gift cohort.

**Methods:** Blood and tissues were collected by rapid autopsy in PWH on suppressive ART (N = 197 sites). Bulk TCR sequencing (Immunovscore TCR kit) was performed across gastrointestinal (GI, 5 sites), central nervous (CNS, 6 sites), lymphoid (6 sites) and vascular systems (2 sites). Data were analyzed using the mixcr bioinformatic platform. HIV reservoir size (HIV DNA) and activity (HIV RNA) was measured by ddPCR. HIV molecular diversity was obtained from single genome env sequencing. HIV integration site (IS) sequencing was performed for one participant in selected tissues. The VDJdb and McPASTCR databases were used to determine TCR specificities. Generalized mixed effects models were used to examine the association between outcomes (proportion of hyperexpanded clones, TCR diversity) with negative binomial for counts, and gaussian for continuous outcomes, and tissue and HIV reservoir measures as predictors.

**Results:** We observed shared clonotypes in all tissues suggesting migration within and across systems, and substantial expansions of TCR clonotypes in all systems. The CNS exhibited more hyperexpanded TCR clones (mean: 45% [34-61]), compared to GI tract (18% [34-61], p = 0.004), lymphoid (13% [8-23], p = 0.001) and vascular (24% [16-38], p = 0.24). The CNS exhibited significantly lower TCR Chao and HIV DNA diversity compared to other systems (Figa-middle right). Higher transcriptional activity (HIV RNA) and HIV DNA diversity in tissues were associated with increased TCR diversity and lower proportion of hyperexpanded clones (p < 0.001). IS sequencing confirmed that proportion of HIV-infected clonally expanded cells was negatively associated with TCR diversity and positively with the proportion of expanded TCR clones. We did not find any evidence for TCR expansions driven by cytomegalovirus or other pathogens.

**Conclusion:** This is the first comprehensive analysis of TCR repertoire landscape across the tissues of PWH. Our findings unveil the expansion and cross-tissue migration of clonal T cells, which are linked to the dynamics of the HIV reservoir. This work sheds light on the interplay between HIV infection and the T cell response, offering insights into the pathogenesis of HIV.

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Minimally Invasive Autopsy Confirms HIV Persistence in Multiple Compartments Despite Prolonged cART
Adriaan Basson1, Nadia Sabet1, Tavneer Omar2, Melanie Moodie1, Monique Nijhuis1, Annemarie M. Wensing1, Caroline Ignacio1, Magali Porrachia2, Caroline Ignacio1, Brandon Woodworth1, Davey M. Smith2, Caroline Ignacio1, Magali Porrachia2, Caroline Ignacio1, Brandon Woodworth1, Davey M. Smith2, David M. Mangoli2, Guochun Jiang2
1University of the Witwatersrand, Johannesburg, South Africa, 2National Health Laboratory Service, Johannesburg, South Africa, 3University Medical Center Utrecht, Utrecht, Netherlands

Background: HIV cure strategies require sophisticated knowledge of the formation and maintenance of latent and active reservoirs of HIV-infected cells. However, the overall paucity of individuals undergoing autopsy have hampered investigation of the anatomical distribution of infected cells during prolonged combination antiretroviral therapy (cART). We report on the largest post mortem study using minimally invasive autopsy on deceased virologically suppressed and unsuppressed adult persons with HIV (PWPH) to characterise viral reservoirs.

Methods: Admitted adult PWH who died at Klerksdorp Theopen Hospital, North West Province, South Africa were included in this study. Relatives of eligible deceased PWH provided informed consent. Causes of death were documented. Ultrasound-guided core needle biopsies were used to sample a broad range of body fluids and organs ≤16hrs after death. Trocars were used for minimal contamination. Organs were verified by histopathological analyses. HIV reservoir analysis from stored fluid and organ samples was conducted by digital droplet PCR, as HIV copies per million cells (cpm).

Results: Sixty recently deceased PWH (38 virally suppressed; 22 unsuppressed of whom 13 were cART-naive) were enrolled with a median time to autopsy of 10.4hrs (IQR 6.9). FIND046 was COVID-19 positive and excluded. Most patients were of Black African descent (94.9%), male (57.6%) 50 y/o (IQR 18), with a median VL<24 RNA copies/ml (IQR 2,401). Virally suppressed PWH were on cART for a median of 66.6 months (IQR 80.3). A total of 489 unique specimens from 11 anatomically distinct compartments were analyzed for HIV viral DNA, including bone marrow aspirate and trephine, brain, bronchioalveolar lavage, cervicovaginal lavage, kidney, lymph nodes, liver, lung, spleen and whole blood. Overall, all anatomical compartments of suppressed and unsuppressed PWH were positive for HIV viral DNA. Highest copy numbers were detected in lymph nodes (7,384±26,695cpm) and lowest copies in brain (513.7±866cpm) and bone marrow trephine (46±314pcm). No statistically significant differences in HIV proviral DNA concentrations between compartments, irrespective of viremic status were noted. However there was an overall trend of higher proviral DNA in all compartments of viremic patients. Sequence analysis showed viral compartmentalization.

Conclusion: Findings provide new insights on anatomical location of HIV-infected cells that persist despite prolonged cART that should be targeted for effective cure interventions.
between infected cells and mock-infected wells (Figure B). This was in contrast with observations of virus-induced cytopathic effects in PBMCs.

**Conclusion:** The long survival of infected BrMCs leads to ineffectiveness of ART, which can be controlled by the addition of HIV transcription inhibitors into the current ART regimen.

![Image](image-url)

**Figure.** (A) ART was less effective in blocking HIV in BrMCs isolated from PWH, which was augmented by a Tat inhibitor Triptolide (Trip) to eliminate HIV production. **p<0.0001** compared with ART. (B) BrMCs sustained cellular viability after infection. No significance compared with mock infection.

### 495 HIV Infection and Reactivation Heterogeneity in Tonsillar and Intestinal Models of HIV Persistence

**Ana Gallego Cortés**, Nerea Sanchez Gaona, Cristina Mancheo Perez, Joan Rey Cano, Oriol Ruiz Isant, Stefania Landolfi, Felix Pumaran, Nuria Ortiz, Inés Llano, Julia G. Prado, Enrique Martín Gayo, Vicenç Falco, Meritxell Genéscà, Maria Buzón
Val d’Hebron Research Institute, Barcelona, Spain, Hospital Universitario de la Vall d’Hebron, Barcelona, Spain, Tübingen Institute for AIDS Research, Badalona, Spain, Universidad Autónoma de Madrid, Spain

**Background:** Tissue reservoirs constitute a significant source of latent and persistent HIV infection in people living with HIV (PLWH) on antiretroviral treatment (ART). While extensive research has focused on understanding HIV reservoirs in the blood, the intrinsic characteristics of viral reservoirs within tissue compartments remain largely unknown. In this study, using tonsillar and intestinal explant models of HIV persistence, we characterized the cellular composition of these anatomical reservoirs and evaluated the effectiveness of latency reversal agents (LRAs) in inducing viral reactivation after ART.

**Methods:** Human tonsillar (n=5) and intestinal (n=7) tissue resections from uninfected donors were ex vivo infected with HIV-1 Bal for 5-6 days, followed by ART treatment for 2 days to establish persistent viral infection. CD4+ T cells were then isolated and cultured for 22 hours in the presence of various LRAs. Using unsupervised clustering analysis (FlowSOM), we identified distinct CD4+ T subsets. We confirmed the presence of viral reservoirs and assessed the effectiveness of LRAs by quantifying intracellular p24 levels after viral reactivation.

**Results:** We identified fifteen CD4+ T cell clusters exhibiting significant inter-tissue variations in the proportions of the majority of these subsets (p<0.05). In both tonsillar and intestinal tissues, viral infection predominantly occurred in various populations of effector memory (TEM: C06, C09, C11 and C14), central memory (TCM: C03 and C07) and T follicular helper (TFH: C10 and C12) cells. However, we found differential enrichment of infected CD4+ T cell subpopulations between tissues (Fig. 1A). Intestinal latently infected CD4+ T cells exhibited greater reactivation with IL15 and AZD5582; whereas the tonsillar subpopulations between tissues (Fig. 1A). Notably, we observed no significant differences in the proportion of these clusters before and after LRA treatment. Furthermore, no significant changes were observed for the LRAs RMD and panobinostat.

**Conclusion:** In our tonsillar and intestinal tissue models of HIV persistence, CD4+ T cell populations exhibited varying susceptibility to viral infection and reactivation, showing significant differences between tissues. Further research is required to identify LRAs effective against distinct HIV cellular reservoirs within these tissues.

### 496 Development of a Single-Cell Multimomic Assay to Phenotype SIV/SHIV Infected Rhesus Macaque Cells

**Jayme M. Nordin**, Vincent H. Wu, M. Betina Pampena, Michael R. Betts
University of Pennsylvania, Philadelphia, PA, USA

**Background:** SIV infection recapitulates many aspects of HIV-1 infection. However, the SIV reservoir at the single-cell resolution remains to be defined. Here, we adapted our single-cell viral assay for Transposase Accessible Chromatin with Select Antigen Profiling by sequencing (viral ASAPseq) to rhesus macaque SIV infection model, to characterize SIV/SHIV reservoir epigenetics and phenotype.

**Methods:** To adapt viral ASAPseq to the nonhuman primate model, we compiled, titrated, and characterized a rhesus cross-reactive antibody panel consisting of 62 immune-related markers using custom and commercially available oligo-tagged antibodies from BioLegend. Using this cocktail, we performed viral ASAPseq on purified lymph node CD4+ T cells at day 13 post-SIVmac251 infection. Finally, we developed a customized bioinformatics analysis pipeline to assess infected cells after viral alignments were made against the SIVmac251 sequence.

**Results:** From this pilot test, we identified 81 SIV+ cells out of 15,334 cells (0.5%); of these, 42 cells had 2 or more reads aligned to multiple regions of the provirus. Using both the epigenetic and surface antigen profiles, we found that 67% of infected cells had characteristics of T follicular helper, T follicular regulatory, or T regulatory cells. The remaining infected cells appeared to have effector, activated, and/or resident memory characteristics.

**Conclusion:** These initial findings demonstrate that NHP viral single-cell ASAPseq can be used to identify SIV+ cells and determine the unperturbed epigenetics and phenotype of these cells. Notably, this assay allows an unprecedented view into the SIV/SHIV reservoir to understand the perturbations associated with various cure strategies.

### 497 Pure CD32+CD4+ Cells Are Cytotoxic Memory CD4+ T-Lymphocytes Highly Enriched for HIV-1 DNA

**Philipp Adams**, Ben Berkhourt, Alexander O. Pasternak
Academic Medical Center, Amsterdam, Netherlands

**Background:** Whether CD32 is a cellular marker of HIV-1 reservoir is a matter of ongoing debate. Despite intensive research, isolation of pure CD32+CD4+ T cells has not been reported thus far. Therefore, the biology of this cellular subset remains ill-defined. Here we present a novel purification strategy that allowed in-depth characterization of this subset in healthy donors as well as in HIV-1 infected individuals.

**Methods:** A purification strategy was developed combining MACS pre-purification of CD4+ T cells with subsequent double FACs sorting, validated by imaging cytometry. Single-cell RNA sequencing and in depth FACs phenotyping was performed followed by unsupervised analysis pipelines. Real time PCR was applied to quantify total HIV-1 DNA.
Persistence of HIV-RNA in Autopsy Tissue Samples From Persons With HIV With Suppressed Viral Loads

Hiromi Iamamichi, Ven Natarajan, Francesca Scrimieri, Mindy Smith, Yunden Badralmaa, Marjorie Bosche, Thomas Buerker, Wei Zhong Chang, Brad Sherman, Kanal Singh, H. Clifford Lane

Background: The tremendous enrichment in HIV-1 DNA suggests that these cellular traits represent an ideal niche for HIV-1 to persist during ART.

Methods: Autopsy specimens were collected from 10 donors: 3 with active HIV-1 replication (blood HIV-RNA levels ≥5 copies/µl), 2 with active HIV-1 replication (blood HIV-RNA levels ≤5 copies/µl), 1 with active HIV-1 replication (blood HIV-RNA levels ≤5 copies/µl), and 3 with suppressed HIV-1 replication (blood HIV-RNA levels <5 copies/µl). HIV-DNA and HIV-RNA species were detected in all 10 donors in 31 different tissue compartments including blood. Levels of HIV-DNA and HIV-RNA species were determined using quantitative PCR. HIV-1 proviruses were analyzed by S 1 LTR-to-3LTR PCR single-genome amplification of near full-length HIV-1 (9 kb in size) and direct amplicon sequencing. A total of 1,902 HIV-1 sequences derived from the 10 donors (median: 128, range: 42 - 697 sequences per donor) were obtained and used for analyses.

Results: HIV-DNA and HIV-RNA species were detected in all 10 donors in 31 different tissue compartments. No statistically significant differences were noted between the Active and Suppressed groups when comparing levels of HIV-DNA in blood or tissues. The same was true for levels of HIV-RNA species in tissues other than blood. The frequencies of full-length intact HIV-1 provirus (encoding replication-competent virus) were similar between the Active and Suppressed groups (5.9% vs. 3.7%, p=0.18). In both populations, the highest levels of HIV-RNA expression were within lymphoid tissues and the ileum. Multiple copies of identical full-length intact HIV-1 provirus were found in one donor in the Suppressed group. The expanded clone was detected in the blood, the kidney and the liver but not associated with HIV-RNA expression.

Conclusion: Outside of peripheral blood, similar levels of HIV-DNA and HIV-RNA expression were noted in autopsy samples from persons with HIV with either active or suppressed HIV-1. These data imply that the substantial seeding of tissues with cells harboring proviral DNA seen in the setting of HIV-1 infection does not change during ART and that many of these cells are actively transcribing HIV-RNA. While it is likely that the majority of this HIV-RNA is coming from cells harboring defective proviruses in the suppressed population, these findings highlight one of the challenges in achieving an HIV cure.

Gut Microbiome Associations With Intestinal HIV Persistence in ART-Suppressed Pediatric Macaques

Nicole Soo, Alexander Grier, Veronika Obregón-Perkó, Zain Gohar Siddiqi, Gloria Mansaré, Bhrugupragyani, Diane G. Camanthian, Julia T. Ng'iro, Rama R. Amarà, Genevieve G. Toukou, Guido Silvestris, Sallie Permar, Ann Chahroudi, Ria Goswami

Methods: Feces were obtained from SHIV.CH505-infected virally-suppressed infant RMs. All RMs received the same triple-drug ART regimen (TFV+FTC+DTG) and were categorized into early ART (n=7, ART start: 4 days post-infection, pi), intermediate ART (n=9, ART start: 2 wks pi), late ART (n=14, ART start: 8 wks pi) and late ART + therapeutic vaccine (n=8, ART start: 8 wks pi, vaccine on ART) groups. The fecal microbiome was profiled using 16s rRNA sequencing. Levels of 21 bile acids and 162 polar metabolites in feces were measured by LC-MS. To determine the impact of microbiome on cell-associated (CA)-RNA, we used Analysis of Compositions of Microbiomes with Bias Correction (bacterial Taxa) and linear regression models (metabolites).

Results: Irrespective of the regimen and duration of treatment, the ratio of SHIV CA-RNA/DNA was higher in the CA-1 T cells of rectal biopsies, compared to blood or lymph nodes. Treatment impacted both fecal-microbial and -metabolomic profiles. After correcting for therapy-based differences, we identified bacterial species Sarcina ventriculi (catabolism) and Treponema spirochetae (virulence) with increased SHIV CA-RNA. Additionally, the levels of Eubacterium hallii, Ruminococcus bromii, Terrisporobacter mayombei, D-glucose, D-glucuronic acid, citric acid, D-fructose and 4-hydroxyproline were inversely correlated with CA-RNA in the GI tract. Finally, a statistical mediation analysis indicated that the association of commensal Eubacterium hallii with reduced viral persistence was mediated via D-glucose.

Conclusions: In the pediatric RM model, gut microbiome appeared to impact intestinal SHIV persistence. Building a GI microbiome from early life that can dampen HIV persistence and prevent reactivation may contribute to the goal of ART-free viral suppression in children living with HIV.
KLR6G1 and CD57 than non-TRM cells in all 3 gut segments, but levels are highest in the colon. CD4+ and CD8+ TRM cell frequencies were negatively associated with total HIV-1 DNA and residual HIV-1 RNA, while non-TRM cells were associated with quasispecies genetic complexity (Figure). Recirculating CD8+ TRM cells were enriched in HIV-1-specific T cells (P<0.01 for IFNγ+, CD107a+, and CFSEdim cells in recirculating TRM vs total CD8+ T cells).

Conclusion: The frequencies of CD4+ and CD8+ TRM cells remained reduced in PLWH on ART, particularly in the colon. Irreversible damage may have been inflicted on these cell populations prior to initiation of ART. The loss of these cells could promote the persistence of HIV-1 in the colon. Enrichment of HIV-1-specific cells in recirculating TRM supports persistent antigenic stimulation by HIV-1 antigens in the gut mucosa of PLWH on suppressive ART.

501 Monocytes/Macrophages Contribute Only Marginally to the Gut Reservoir of HIV-1 on ART
Camille Vellas1, Mary Requena1, Manon Nayrac1, Karl Barange1, Laurent Alric1, Guillaume Martin-Blandet1, Jacques Izopet1, Pierre Delobel2
1Toulouse University Hospital, Toulouse, France, 2Centre de Recherche du CHUM, Montreal, Canada

Background: Current antiretroviral treatments (ART) are unable to cure HIV-1 infection due to the persistence of latently infected cells. The gut mucosa contains numerous target cells, and high levels of HIV-1 DNA persist in this compartment under ART. CD4+ T cells are the best-characterized reservoir for HIV-1. However, macrophages are abundant long-lived cells in the gut, but their involvement in HIV-1 persistence under ART remains debated.

Methods: To investigate the contribution of intestinal monocytes/macrophages to the HIV-1 reservoir, we collected duodenal (N=8) and colonic (N=8) biopsies from 12 people living with HIV (PLWH) under suppressive ART (viral load <30 copies/mL) for 8 years, included in the ANRS EP61 GALT study. T cells were isolated by positive CD2 magnetic sorting, and monocytes/macrophages were sorted by flow cytometry. Cellular DNA was extracted and amplified by REPLI-g. We quantified total HIV-1 DNA by qPCR in the LTR region and integrated HIV-1 DNA was detected in a third of T cell and monocytes/macrophages positive samples. Using the IPDA assay, we detected intact HIV-1 proviruses in 4/16 of T cell samples but in only 1/6 of monocyte/macrophage samples.

Results: Total HIV-1 DNA levels in intestinal T cells (positive samples: 8/8 in the duodenum and 8/8 in the colon, median 3,398 copies/10^6 CD4+ T cells [IQR, 1,208-12,312]) were much greater than those in monocytes/macrophages (positive samples: 4/8 in the duodenum and 3/8 in the colon, median 12 copies/10^6 monocytes/macrophages [IQR, 8-22] (P<0.001). Unintegrated HIV-1 DNA was detected in a third of T cell and monocytes/macrophages positive samples. Using the IPDA assay, we detected intact HIV-1 proviruses in 4/16 of T cell samples but in only 1/6 of monocyte/macrophage samples.

Conclusion: We showed that monocytes/macrophages from the intestinal mucosa of both the duodenum and colon of PLWH under suppressive ART can contain HIV-1 DNA, even intact or unintegrated, but at much lower levels (300-fold lower) than those found in T cells. These findings provide further evidence that monocytes/macrophages contribute only marginally to the HIV-1 reservoir in the gut of ART-treated individuals.

Background: While HIV-1 group M is responsible for the large majority of HIV infections worldwide, 3 others groups named O, N and P (HIV-1 non-M) are more genetically divergent and concentrated in West-central Africa with sporadic cases in Europe, America and Canada. Previous works have demonstrated the limited number of therapeutic options due to the natural genetic polymorphisms associated with HIV-1 non-M. Thus, our work aimed to determine the in vitro susceptibility of these particular strains to Ibalizumab, a first-in-class long-acting CD4-directed post-attachment inhibitor.

502 Non-Invasive Imaging Identifies Elevated Metabolic Activity in Lymphoid Tissue During Long-Term ART
Chuen-Yen Lau, Jessica Earhart, Danielle Konlian, Ariana Savramis, Thuy Nguyen, Corina Millo, Avindra Nath, Robert Gorelick, Ana Ortega-Villa, Michael Kassin, Elliott Levy, Brad Wood, Dima Hammoud, Frank Maldarelli, for the NIH Biopsy and Treatment Interruption Team
National Institutes of Health, Bethesda, MD, USA

Background: Mechanisms of HIV persistence in anatomical compartments remain poorly understood. Positron Emission Tomography (PET) is a non-invasive technique that may provide insights to the spatial distribution and microenvironment of HIV persistence and identify sites for tissue sampling. Early 18F-fluorodeoxyglucose PET (FDG-PET) techniques did not detect elevated metabolic activity during long-term antiretroviral therapy (ART), though increased metabolic activity was observed with rebound viremia. To investigate whether new technologically advanced PET imaging with improved resolution detects metabolic activity in lymphoid tissues during long-term ART, we used FDG-PET co-registered with computed tomography (CT) followed by fusion guided lymph node biopsy to obtain samples for characterization.

Methods: Participants recruited through NIH study NCT04519024 (Imaging and Biopsy of HIV-Infected Individuals Undergoing Analytic Treatment Interruption (ATI)) underwent FDG-PET-CT and FDG uptake was quantified as standardized uptake value (SUV; (FDG uptake normalized to injected activity/weight)). Tissue was sampled with ultrasound fusion guidance and aspiration. HIV RNA in plasma and cell-associated (CA) HIV DNA and RNA in cells from blood and lymphoid tissue were quantified by single copy assays (Somouk et al., 2014). Results: Study participants (N=2; HIV RNA <50 copies/mL plasma for >3 y, CD4 562-590) underwent imaging and guided biopsy. Imaging noted lymph nodes were all < 1 cm diameter; although most nodes had minimal FDG signal, increased uptake was detected in c. 3-10 lymph nodes in both participants (SUVmax 1.14 - 6.4), indicating increased metabolic activity was present in these tissues. Metabolically active nodes were present in mediastinal, axillary and inguinal distributions. Nodes accessible to fusion guided sampling yielded 104-165 cells. Levels of HIV DNA in biopsies (610-900 HIV DNA copies/16x cells) were c. 5-15-fold higher than levels in peripheral blood mononuclear cells (PBMC). HIV RNA was quantifiable in both nodal tissue and PBMC; levels of HIV RNA/DNA (0.4-1.2 RNA copies/DNA copy) were comparable in blood and nodal sampling.

Conclusion: New non-invasive imaging approaches identify increased metabolic activity in subsets of lymph nodes during long-term ART, and directed biopsy recovers sufficient HIV DNA and RNA for analysis even in nodes < 1 cm size. Image guided approaches will advance understanding of HIV persistence in tissues.

503 Inducible, Infectious HIV-1 Resistance to Autologous Neutralizing Antibodies After Long-Term ART
Natalie F. McMyn, Joseph Varriale, Hanna Wu, Janet M. Siliciano, Robert F. Siliciano
The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: ART reduces HIV-1 viral loads below the detection limit, but ART cessation leads to rapid viral rebound due to a population of latently infected CD4+ T cells carrying inducible, replication-competent proviruses. Current HIV
cure strategies aim to delay or prevent viral rebound. Previously, we showed that autologous neutralizing antibodies (aNAb) can prevent ex vivo outgrowth of some viruses in the latent reservoir of people with HIV (PWH; Bertagnolli et al., PNAS 2020) and that the reservoir remains stable for decades (McMyn et al., JCI 2023). However, it is not understood whether viruses persisting over long times on ART are sensitive to aNAb.

**Methods:** Using samples of outgrowth viruses from nine PWH on long-term ART for a mean of 22 years and four PWH on ART for 11 years, we generated HIV pseudoviruses with the env sequences of inducible, replication-competent outgrowth viruses. Pseudoviruses of viral variants from each person were incubated with contemporaneous aNAb and tested in a TZM-bl-based neutralization assay. Viral variants were also tested against three clinically relevant broadly neutralizing antibodies (bNAb) targeting different neutralizing epitopes.

**Results:** Most outgrowth viruses from PWH on long-term ART were resistant to neutralization by bNAb, with 85% of 34 isolates being resistant (IC50 >100 ug/ml). Conversely, PWH on ART under 20 years had more sensitive isolates (IC50; 3 to 99 ug/ml), as only 22% of 55 viral isolates were resistant to aNAb (IC50 >100 ug/ml). Viral isolates from both groups demonstrated similar sensitivities to the three bNAb (VRC01, 10-1074, PGDM1400; IC50 <0.3 to >0.3). 89% of PWH on long-term ART had all tested viral isolates sensitive to at least one bNAb and 56% sensitive to two bNAs, while 100% of PWH on short-term ART were sensitive to at least one bNAb and 50% sensitive to two bNAs.

**Conclusion:** We demonstrated that in PWH on long-term ART, outgrowth viruses including large clones are more resistant to neutralization by bNAb than proviruses induced in CD4+ T cells of PWH on ART for 11 years. This suggests a selection process may occur over two decades of ART. These aNAb resistant outgrowth viruses may contribute to viral rebound during treatment interruption, but this data may inform cure strategies with therapeutic vaccines that induce antibodies to neutralization-resistant viruses. Additionally, the large proportion of viral isolates sensitive to at least one bNAb could aid in the design of immunotherapy trials involving bNAb.

**Immune Selection of HIV-1 Reservoir Cells After Early ART Initiation**

Weiri Sun, Gregory Gladkov, Le Gao, Leah Carrere, Isabelle Roseta, Elizabeth Parsons, Carmen Garca Capote, John Frater, Sarah Fidler, Xu G Yu, Mathias Lichterfeld.

**Background:** HIV reservoirs are established shortly after infection, but little is known about immune effects that influence viral reservoir cell evolution in early-treated people living with HIV (PLH). Here, we analyzed the proviral landscape and phenotypic features of HIV reservoir cells in the RIVER study, a randomized-controlled study evaluating effects of a ChAd63-vectored vaccine, in early-treated people living with HIV (PLH). Here, we analyzed the proviral landscape and phenotypic features of HIV reservoir cells in the RIVER study, in early-treated people living with HIV (PLH). Here, we emphasized the phenotype of memory CD4 T cells from 3 participants.

**Methods:** 10 RIVER study participants were studied, using PBMC samples from randomization, the primary endpoint (18 weeks after randomization), and 1 year after study completion. Proviral landscapes were analyzed by next-full-length proviral sequencing (FLIP-seq) and matched integration site and proviral sequencing (MIP-seq). Phenotypic and proviral sequencing (PheP-seq) was used to investigate the phenotype of memory CD4 T cells from 3 participants.

**Results:** In total, n=2763 proviral sequences were detected from 122.03 million PBMCs in all 10 participants combined; n=295 sequences (10.68%) were genome-intact. The mean numbers of intact HIV proviruses from randomization, primary endpoint and 1 year follow-up were 6.27, 2.39, 8.84 per million PBMC in the ART-only group versus 4.42, 2.37, and 5.46 per million cells in vaccine group (p=n.s.). 138 integration sites (IS) of intact proviruses were identified; among these, 54 (39.13%) were located in non-genic DNA, centromeric satellite DNA and genes encoding for members of the Zinc Finger Protein family. The proportion of intact proviruses integrated in these heterochromatin regions increased during longitudinal evaluations from 26% at randomization to 54% at the 1-year follow-up timepoint, but did not differ between the study arms. A total of n=116,023 individual mCD4 T cells were analyzed by PheP-seq: n=648 represented HIV-infected cells and n=128 cells harbored genome-intact HIV-1. Notably, HLA-G, HLA-F, HLA-C, CCR6 and TFGB-R were upregulated on HIV-1 reservoir cells with intact proviruses, consistent with immune selection primarily mediated by innate immune responses.

**Conclusion:** These data suggest accelerated and more efficient immune selection of HIV-1 reservoir cells when ART is started during early disease. Immune selection appears to be predominantly driven by innate immune responses in early infection, while the therapeutic T cell vaccine had no detectable effect.

**Identification and Targeting of Metabolic Profiles in CTL-Resistant, HIV-Infected CD4+ T-Cells**

Alberto Herrera, Louise Leyre, Jared Weiler, Paul Zumbo, Doron Betel, R. Brad Jones

Weill Cornell Medicine, New York, NY, USA

**Background:** HIV-specific CTL responses remain present even under long-term ART, in association with residual HIV expression. Selection of CTL-resistant reservoir-harboring clones may contribute to HIV persistence, with BCL-2 overexpression being a previously reported mechanism. To identify additional mechanisms of resistance, we developed an in vitro model that compares infected cells surviving CTL pressure to non-targeted infected bystander cells in the same environment. Here, we highlight a specific metabolic profile enriched in CTL-resistant infected cells.

**Methods:** CD4+ T-cells were infected with HIVJRSCF (WT) or a variant containing an escape mutation in the Gag-TW10 epitope (TW10esc). Killing assays were performed by labeling infected T-cells with CTRF (WT) or CFSE (TW10esc) dyes, then culturing these together with a TW10-specific CTL clone. Surviving WT-infected and bystander TW10esc-infected cells were sorted based on HIV-Env expression and profiled by RNAseq, CITeseq and flow cytometry. Killing assays were also performed with pre-treatment of infected cells with the FDA approved antimalarial Atovaquone (ATQ) and iron chelator Deferoxamine (DFO).

**Results:** WT-infected survivors exhibited distinctive transcriptional and protein expression profiles, relative to bystanders (Figure 1). GSEA analysis of RNAseq data revealed that among several dysregulated pathways, WT-survivors were negatively enriched for Hallmark Glycolysis (n=4, NES = -1.74, Padj = 2.96e-05) and byproducts of active metabolism such as Hallmark Hypoxia (NES = -1.73, Padj = 1e-05). Flow cytometry on WT-survivors showed enrichment of cells expressing lower levels of cellular reactive oxygen species (ROS) (n=4, mean = -24.41% +/- 13.62, P=0.037). Pre-treatment of infected cells with ATQ, to increase ROS accumulation, and DFO, to induce hypoxia, modestly and robustly enhanced susceptibilities of infected cells to elimination by CTL respectively (ATQ: n=5, P = 0.09; DFO: n=4, P = 0.015; ATQ+DFO: n=4, P = 0.051).

**Conclusion:** Oxidative disbalance contributes to CTL mediated killing. Our results suggest that lower levels of ROS and hypoxic responses in HIV-infected cells with particular metabolic features renders these cells less susceptible to killing by CTL. Treatment with FDA-approved and well-tolerated ROS inducer ATQ and hypoxia-inducing iron-chelator DFO increased CTL mediated elimination of infected cells in vitro. Targeting metabolic balance may be a strategy to enhance elimination of persistent HIV-infected cells.

**Immune Profile During ATI in AELIX-002 HTI Vaccine Trial and Its Role in Post-Intervention Control**

Cristina Peligero-Cruz, Lucia Bailon, Samandhy Cedeño, Jose Molto, Beatriz Mothe, Aelix Therapeutics, Barcelona, Spain

**Conclusion:** High titers of autologous neutralizing antibodies (aNAb) are able to apply selective pressure on rebounding viruses after ART interruption (ATI). Recent
findings in CHAMP cohort also show higher frequencies of activated natural killer (NK) cells in post-treatment controllers (PTC). In AELIX-002, a randomized controlled clinical trial testing HTI T-cell vaccines in early-treated PWH, all participants experienced detectable viral rebound during ATI, but lower viremia and longer ART-free periods (>12 weeks) were observed in vaccine recipients in whom vaccination induced robust cytotoxic HTI responses pre-ATI. Here, we explore humoral, innate, and T cell responses during ATI.

Methods: We used plasma and PBMC from 41 AELIX-002 participants (1) before ART was initiated (pre-ART), (2) at study entry (BL), (3) at first recrudescence timepoint (ATI-Rc), at peak viremia (ATI-Pk), (5) at viral setpoint (ATI-Setp) and (6) at the end of ATI (ATI-end). Neutralizing antibodies were measured against a panel of 6 HIV-1 pseudoviruses (Tier 1 and 2) and the pre-ART autologous virus in a standard TZM-bl cell-based assay. T, B, and NK cell composition, activation and exhaustion were measured by flow cytometry. Total HIV- and HTI-specific responses were measured by IFNγ ELISPOT.

Results: Pre-ART, only three participants showed low-to-moderate neutralization of NL4-3 pseudovirus, and one participant, of the autologous virus. At ATI-Pk, 27% placebo and 4% vaccinees neutralized NL4-3, while 21% placebo and 12% vaccinees had detectable neutralization to the pre-ART autologous virus. Despite viral recrudescence occurring in all participants, vaccinees who remained off ART > 12 weeks had stronger and more HTI-focused responses at ATI-end compared to the rest of participants and presented a unique immunological profile characterized by i) lower levels of plasma B cells, ii) lower levels of activated B and CD8 T cells, and iii) less exhaustion after peak viremia and up to ATI-end. Unlike PTC, no increase in activated NK was seen in vaccinees who remained off ART for >12 weeks at any timepoint.

Conclusion: After HTI-vaccination, the immune profile of participants remaining off ART for >12 weeks was different from those described in other studies on post-treatment controllers. While no significant contribution of humoral and innate responses was detected, durable HTI-specific responses and lower B- and T-cell activation profiles were maintained during ATI despite viral recrudescence.

507 IL-1β Blockade in PWH on ART Enhanced Host Antiviral Responses and Cytotoxic Effector Functions

Suligii A. Lee1, Ashish A. Sharma2, Naseem Sadek1, Danny L1, Ashok K. Dwivedi1, Rachel L. Rutishauser1, Steven G. Deeks1, Rafick P. Sekaly2, Priscilla Y. Hsu1, Jeffrey A. Tomalika1
1University of California San Francisco, San Francisco, CA, USA; 2Emory University, Atlanta, GA, USA

Background: Plasma IL-6 levels are amongst the strongest predictors of mortality in people with HIV (PWH), and in IL-1β, an upstream regulator of IL-6, may be the major driver of this risk. In vivo IL-1β blockade with the monoclonal antibody, canakinumab, significantly reduced arterial and bone marrow inflammation in our prior pilot study of PWH on ART. Here we now identify the host immune mechanisms associated with IL-1β blockade, using single cell mass cytometry (CyTOF) and transcriptomic approaches.

Methods: A total of 10 PWH on ART with known cardiovascular disease (CVD) or 1 traditional CVD risk factor, were administered a single subcutaneous dose of canakinumab, among 6 “virologic responders” (no change in HIV total DNA). Single cell sequencing revealed significantly lower levels of the exhaustion markers (TOX, TIGIT and LAG3) in CD8+ T cells in virologic responders, although levels of granzyme and perforin were similar between groups.

Conclusion: Our multiomic analysis highlights three potential novel mechanisms to target HIV after in vivo IL-1β blockade: (1) augmenting antiviral immunity, (2) enhancing cytotoxic CD8+ T cell effector function, and (3) reversing dysregulated immune activation of CD4+ T cells. Thus, IL-1β, via a multi-faceted approach, may induce an immunologic milieu favorable for HIV reservoir clearance. The figure, table, or graphic for this abstract has been removed.

508 Elevated Plasma IL-10 and Type I Interferon Predict Faster HIV Reservoir Decay in Acute Treated HIV

Lei Shi1, Junzhe Shao1, Sannidhi Saravadasvaimuthu1, Maria Sophia B. Donaire1, Alton Barbeahmen1, Rebecca Holt2, Gregory M. Laird3, Frederic Hecht4, Christopher Pilcher5, Timothy J. Henrich5, Jingshen Wang1, Jeffrey A. Tomalika1, Rafick P. Sekaly6, Steven G. Deeks7, Suligii A. Lee1
1University of California Berkeley, Berkeley, CA, USA; 2University of California San Francisco, San Francisco, CA, USA; 3Acceleve Diagnostics, Baltimore, MD, USA; 4Emory University, Atlanta, GA, USA

Background: The HIV reservoir largely consists of “defective” virus, but the elimination of the “intact” (replication-competent) reservoir is a major focus of HIV eradication strategies. There are limited data describing reservoir decay rates during the first few months of acute-treated HIV, nor the host immune responses that drive reservoir decay. We quantified plasma cytokine levels from >500 longitudinal samples from the UCSF Treat Acute HIV cohort to determine immunologic pathways that predict reservoir decay in people with HIV (PWH).

Methods: Individuals diagnosed with acute (<100 days) HIV were enrolled between 2015-2020, immediately initiated on ART (tenofovir/ emtricitabine+raltegravir), and followed monthly for the first 24 weeks of ART initiation. Frequencies of intact vs. defective provirus were quantified using the IPD4. High-sensitivity multiplex plasma cytokine assays were performed from cryopreserved plasma samples (Mesoscale Diagnostics). Multivariate nonlinear general additive models were adjusted for false discovery rate (FDR) using the Benjamini-Hochberg method.

Results: Among 67 PWH diagnosed <100 days from HIV acquisition, we observed an initial rapid (<8 weeks) decay, followed by second slower (8-24 weeks) decay of both intact and defective HIV proviral DNA during the first 24 weeks of ART. Among 20 plasma cytokines assayed across these same longitudinal timepoints (weeks 0, 2, 4, 8, 12, 16, 20, 24), IL-10 and type I interferons (IFN-β) significantly predicted accelerated reservoir decay even after adjustment for initial CD4+ T cell count, pre-ART viral load, age, and timing of ART initiation. IL-10 was significantly associated with faster decay rates for intact, but not defective virus, during both phases of decay (5.74% and 1.32% increase in decay rate/week per unit-increase in IL-10, respectively). IFN-β was significantly associated with faster decay rates during the second decay phase (0.89% intact and 1.55% defective HIV DNA).

Conclusion: Individuals with higher plasma IL-10 and type I IFN expression during the first 24 weeks of ART demonstrated accelerated HIV reservoir decay. These cytokines are well known to exert variable effects on the host immune response depending on stage of disease (e.g., favorable during acute but detrimental during chronic infection). Our findings add insight into the complexity of these pleiotropic cytokines and highlight the need for potential stage-specific taretining of these cytokines in future HIV cure strategies.

Figure 1. Among 20 cytokines assayed, IL-10 and IFN-β statistically significant predicted first (A) and second (B) phases of HIV reservoir decay. Bars=95% confidence intervals. Red font=statistically significant in multivariate nonlinear models adjusted for initial CD4+ T cell count, pre-ART viral load, age, and timing of ART initiation (FDR q<0.05).

A. First Phase of Decay (0-12 Weeks ART)
B. Second Phase of Decay (12-24 Weeks ART)

509 Labelled High Affinity TCRs for Detection of HIV Epitopes on Infected Cells: How Low Can We Go?

Zoe Wallace1, Jonathan Chamberlain1, Esra Guc1, Praveen K. Singh1, Lucy Dorrell2, Alton Immunocore, Abingdon, United Kingdom

Background: HIV proviruses persist in a non-productive state in CD4+ cells. There is no individual cell surface biomarker that uniquely identifies infected
cells. However, some cells contain proviruses that are transcriptionally active and translationally competent, leading to expression of viral proteins and T cell epitopes. We used soluble affinity-enhanced T cell receptors (TCR) to investigate the level of expression of viral peptides presented by HLA class I molecules.

**Methods:** We developed T cell receptors with picomolar affinity (µM) for an HIV Gag epitope presented by HLA-A*0201 and incorporated either a biotinylation tag or Fc fragment to enable fluorescence imaging. CD4+ T cell lines or primary cells with varying HLA expression levels were pulsed with peptide or infected with HIV and analyzed for expression of viral peptide-HLA complexes by total internal reflection microscopy (TIRF; single molecule epitope counting).

**Results:** HIV Gag peptide-HLA complexes could be identified on peptide pulsed cells using a labelled TCR and TIRF microscopy. The signal/noise ratio was improved using the TCR-Fc format. T2 cells pulsed with a titration of peptide were then used to quantify phGILA copies/cell and determine the dynamic range of the assay (~10–200 phGILA/cell). phGILA could also be detected on the surface of HIV-infected HLA-A*0201-transduced CB166 cells, albeit at copies/cell near the lower limit of detection of the assay. Similar results were obtained with primary CD4+ T cells infected in vitro with HIV. In parallel we showed that the same TCR, when used in a bispecific format (Gag x CD8) could eliminate infected CB166 cells at picomolar drug concentrations in a T cell redirection assay.

**Conclusion:** An affinity-enhanced TCR targeting a Gag peptide successfully detected phGILA complexes on the cell surface down to ~10 copies/cell and could redirect killing of infected cells when used in a bispecific format despite low phGILA expression, demonstrating its high sensitivity to antigen. Further work is ongoing to develop flow cytometry applications using labelled high affinity TCRs with the potential to isolate HIV-infected cells ex vivo for further analysis.

**510 HIV T-Cell Immunity Predicts Intact Provirial DNA Decline in People Treated During Acute Infection**

Pien M. van Paassen1, Alexander O. Pasternak1, Ninée V. Buchholz2, Karel A. van Dort3, Michelle J. Knouwen4, Liffert Vogt5, Casper Roake5, Tokamah Mahmoudi6, Cynthia Lunga7, Jori Symons8, Monique Nijhuis9, Jan M. Prins10, Neelie Kootstra11, Godelieve J. de Bree12

1 Academic Medical Center, Amsterdam, Netherlands, 2 University Medical Center Utrecht, Utrecht, Netherlands, 3 Erasmus University Medical Center, Rotterdam, Netherlands.

**Background:** Starting antiretroviral therapy (ART) during acute HIV infection (AHI) is known to limit damage to the immune system and lower the size of the viral reservoir. In light of HIV cure interventions, it is important to understand the longitudinal dynamics of the early host immune responses in relation to the viral reservoir size. Therefore, we investigated the viral reservoir size and HIV-specific immune responses in participants of the Netherlands Cohort Study on Acute HIV Infection (NOVA study), who initiated ART immediately after diagnosis of AHI.

**Methods:** Participants in the NOVA study diagnosed during Fiebig II-VI were included in the analysis (n=22). PBMC from leukapheresis at 24 and 156 weeks after initiation of ART were analyzed. Viral reservoir size was assessed by Intact Provirial DNA Assay and qPCR. HIV specific precursor T-cell responses after HIV peptide pool stimulation (Env, Gag, Nef, Pol) were determined by flow cytometry. Correlations were determined using Pearson’s correlations (p<0.05).

**Results:** A mean decline in intact proviral DNA (Fig.1A) and total HIV-DNA (10-13%) between 24 and 156 weeks after ART initiation was observed. We measured a positive HIV specific CD4+ and CD8+ T cell response to at least 3 different viral proteins in the majority of individuals at both time points (Fig.1 B+C). At 24 weeks, intact proviral DNA load was associated with CD4+ T cell responses to Env (p<0.001, R²=0.96), Gag (p=0.005, R²=0.6), Nef (p=0.001, R²=0.9) and Pol (p=0.002, R²=0.8). At 156 weeks, intact proviral DNA load was correlated with CD8+ T cell response to Gag (p=0.034, R²=0.59) and Pol (p=0.001, R²=0.77). Moreover, we observed a positive association between the decay of intact proviruses between 24 and 156 weeks with CD4+ T-cell responses to Env (p=0.034, R²=0.64) and Nef (p=0.007, R²=0.76). No associations between T-cell responses and other viral reservoir measurements (defective proviral DNA load, total HIV-DNA and US-RNA) were observed.

**Conclusion:** We detected a positive association between HIV specific T-cell responses and the decay of intact proviral DNA load in individuals treated during AHI. This implies that a potent HIV specific T-cell response, in addition to early ART, leads to a reduction of intact proviral DNA load. This finding potentially provides a promising avenue for cure interventions aimed at reservoir induction in combination with T-cell activating strategies. The figure, table, or graphic for this abstract has been removed.
the neutralization curve (-0.029 change in log10 [Y/a/(1-Fa)]) per log10 38NC17 concentration, 95% Confidence Interval: -0.049; -0.010, P = 0.003). Among the three bNAb sensitivity assays, the slope of the logistic was the best predictor of second-phase viral decay outperforming any IC values or genotypic assessments. The sensitivity and specificity in predicting a faster second-phase viral decay with a slope of 2.25 was 62% and 75%. The best next predictors of plasma viral decay in the second phase were IC50 <1.2 µg/mL with sensitivity and specificity of 78% and 63% on the PhenoSense assay and a threshold for single envelope sequences of 75% on the bNAb-REP (90% of all sequences needed to be categorized as sensitive) with a sensitivity and specificity of 77% and 75%.

Conclusion: Whilst bNAb sensitivity is crucial for clinical outcome in clinical trials administering bNAbS, so far bNAb sensitivity threshold has been based on expert opinions. Using clinical data from the eCLEAR trial, we have shown that the best predictors of bNAb-mediated effects on plasma HIV-1 RNA decay are the slope of the neutralization curve from the PhenoSense assay.

513 HIV-1 Reservoir Dynamics in Children With Early Treated, Perinatally-Acquired HIV: Does Sex Matter?

Kavirda Reddy1, Chantal Molechon1, Moira J. Spyker2, Mathias Lichterfeld1, Pablo Rajoj3, Paolo Ross1, Paolo Palma3, Carlo Giaquinti3, Aafia Liberty3, Moruy Isaacs4, Loide Cardoso5, Ligia Esteva6, Alfredo Tagaro7, Thomas Hilding5
1Africa Health Research Institute, Durban, South Africa, 2University College London, London, United Kingdom, 3Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, 4Hospital Universitario de Octubre, Madrid, Spain, 5Bambino Gesù Children’s Hospital, Rome, Italy, 6University of Padova, Padova, Italy, 7University of the Witwatersrand, Johannesburg, South Africa, 8Stellenbosch University, Cape Town, South Africa, 9Fundación Alvaro de Diéses, University of the Witwatersrand, Johannesburg, South Africa.

Methods: HIV DNA copy number and proviral load at ART initiation were measured in total PBMCs by droplet digital PCR from 34/52 infants, 18 females and 16 males, termed early treated HIV-infected children. The timing of ART initiation during acute infection was determined by Fiebig stage. We compared 4 years of follow-up. Results: ART was initiated at a median of 34 days of life (IQR, 26.0-73.0) and the median time to viral suppression in virological controllers was 363 days on ART (IQR, 317.0-552.0). There was a positive correlation with baseline viral load and proviral load each measured at ART initiation (p=0.03, r=0.58) and 1 year (p=0.01, r=0.56) and 2 years (p=0.01, r=0.69) of follow up. At ART initiation the median total HIV DNA level was 3.7 log copies/million cells (IQR, 3.2-4.1). The HIV DNA reservoir decreased slowly over the first 6 months (median = 3.2 log c/10^6 cells, IQR, 2.6-3.7) of ART with significant decline after 1 year (median = 2.6 log c/10^6 cells, IQR, 2.3-3.1) (p=0.002). There was a further steady decline over the next 3 years (median = 2.05 log c/10^6 cells, IQR, 0.5-2.7) (p<0.0001). However, total HIV DNA remained detectable in 9/12 (75%) participants after 4 years. There was no difference in proviral load at ART initiation between sexes but after 4 years the HIV reservoir size decreased significantly in males (p=0.006) but not in females.

Conclusion: Decline in total HIV DNA is observed after 1 year of treatment and is still detectable after 4 years of treatment. The faster reservoir decay in males is novel, suggesting that sex differences should be considered to optimize HIV cure strategies in children.

514 Earlier Initiation of ART Reduces Intact Proviruses but Not Residual Viremia After 48 Weeks

Joshua C. Cytker1, Joseph Palemo2, Gregory M. Laird1, Dianna Hoeth3, Justin Ritz3, Albine Martin1, Gert U. van Zyl4, Eric Daar2, Trevor A. Crowell4, John J. Eron2, Albine Mellors1, for the ACTG A5354 Team
1University of Pittsburgh, Pittsburgh, PA, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3Academic Diagnostics, Baltimore, MD, USA, 4Stellenbosch University, Cape Town, South Africa, 5Harbor-UCLA Medical Center, Tustin, CA, USA, 6Henry M Jackson Foundation, Bethesda, MD, USA, 7University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Background: The timing of antiretroviral therapy (ART) initiation during acute or early HIV infection (AEHI) may affect the size of the latent and expressed HIV reservoir but its specific influence on intact proviral DNA (IPD) or low-level viremia has not been fully described.

Methods: AIDS Clinical Trials Group Study A5354 enrolled 192 adults who started ART during AEHI at 30 sites in the Americas, Africa, and Asia. Participants were retrospectively centrally categorized as Group 1 (Fiebig I-II), Group 2 (Fiebig III-IV), or Group 3 (Fiebig V) and measures of HIV persistence were evaluated on a subset of 106 US study participants or those with confirmed subtype B. The intact proviral DNA assay (IPDA*) was performed on CD4+ T cells isolated from peripheral blood at weeks 24 (not shown), 48, and 72 after ART initiation. Plasma HIV RNA was measured by automated single copy assay (SCA) at week 48. Wilcoxon rank-sum and Fisher’s exact test were used for pairwise comparisons between Groups and Jonckheere-Terpstra test for trends across the 3 Groups.

Results: Participants had a median (IQR) age of 29 (24, 39) years, 11 (10%) were female, 3 (3%) identified as transgender, 58 (57%) were Black, and 103 (97%) initiated a regimen of EVG/COBI/FTC/TAF. Participants in Group 1 had a nominally lower level of total proviral DNA (IPD + defective DNA) than Groups 2 and 3 at weeks 24 and 48 (p=0.038). Participants who started ART during the earliest study groups had significantly lower IPD levels at all three timepoints (trend tests p=0.033) but with substantial variation and overlap in IPD copies per million CD4+ T cells (Figure 1). No significant longitudinal trends were observed in the decline of IPD across Groups. Persistent plasma HIV RNA was detected by SCA in >75% of participants at week 48 with no significant differences in the proportions with detectable HIV RNA (76-85%; p≤0.041) or the levels of HIV RNA between pairwise groups (p≤0.22). IPD and plasma HIV RNA at week 48 were significantly but modestly correlated (r=0.42, p<0.001).

Conclusion: Here we show that earlier ART lowers the levels of total and IPD in Groups 1 and 2 (Fiebig I-V) compared with Group 3 (Fiebig V). Surprisingly, residual plasma HIV RNA was detected in >75% of participants at week 48 with no significant differences in proportions or levels between study Groups. These results provide new evidence that earlier ART does not affect the active HIV reservoir measured by plasma HIV RNA.

515 Host and Viral Factors Shape the Composition of the HIV-1 Viral Reservoir in the 2000HIV Cohort

Mareva Delport1, Kavita Mehta2, Maxime Verschoore3, Elizabeth R. Wonderlich4, Wilhelm A. Vos5, Albert L. Groenendijk6, Louise E. van Ekeren7, Marc Blaauw8, Evi E. Blomme9, Sofie Ruusart9, Sarah Gerlo9, Wim Trypsteen9, Mihai Neute9, Andre J. van der Ven10, Linos Vandekerckhove11
1Ghent University, Ghent, Belgium, 2HIV Healthcare, Brentford, United Kingdom, 3OIVG, Amsterdam, Netherlands, 4Erasmus University Medical Center, Rotterdam, Netherlands, 5Rainbow University Medical Center, Nijmegen, Netherlands.

Background: The HIV-1 host interaction exhibits remarkable diversity, resulting in various clinical profiles and virological traits, including coreceptor tropism. The 2000HIV study, which is a multi-omics study including 1895 people resulting in various clinical profiles and virological traits, including coreceptor tropism. The 2000HIV study, which is a multi-omics study including 1895 people.

Methods: We quantified the total and intact HIV-1 reservoir by using the Rainbow proviral HIV-1 DNA digital PCR assay in CD4+ T cells from blood in a subset of PLWH from the 2000HIV cohort (n = 863). In this study, we identified two distinct groups of PLWH, LoViReT-like and HiViReT-like, based on their coreceptor tropism. The 2000HIV study, which is a multi-omics study including 1895 people.

Results: Quantity of the Rainbow HIV-1 DNA assay resulted in a median of 4561 (95% CI: 410.2-521.4) total HIV-1 DNA copies/10^6 CD4+ T cells (n =
Conclusion: Earlier ART initiation was associated with lower levels of intact proviral DNA in PBMCs that persisted through 10–15 years of suppressive ART, at which point they were about 30% smaller in the early-treated group. Significant reservoir decay was observed through 10 years of ART. Future studies of curative interventions may consider enrolling participants who initiated ART within one year of estimated HIV acquisition, particularly those with a long history of suppressive ART.
518 Tumor Suppressor and Innate/Inflammatory Pathways are Associated With the HIV Reservoir Size

German G. Gornalusse,1 Ashok K. Dwivedi,2 Rebecca Hoh,2 Jeffrey Martin,3 Frederick Hecht,4 Meei-Li Huang,1 Julieta Repetti,1 Phuong M. Vo,1 Claire M. Levy,1 Pavitra Roychoudhury1, Keith R. Jerome,4 Florian Hladik,5 Timothy J. Henrich,6 Steven G. Deeks,5 Subagga A. Lee5

1University of Washington, Seattle, WA, USA, 1University of California San Francisco, San Francisco, CA, USA, 2University of Buenos Aires, Buenos Aires, Argentina

Background: The major barrier to an HIV cure is the HIV reservoir: latently-infected cells that persist despite effective antiretroviral therapy (ART). Most prior studies of host genetic predictors of HIV control have focused on “elite controllers,” rare individuals able to control virus in the absence of ART. However, there have been few genetic studies among ART-suppressed non-controllers, who make up the majority of people with HIV (PWH).

Methods: We performed a large cross-sectional study of 191 PWH on ART and measured host gene expression (RNA-seq) and HIV reservoir from peripheral blood CD4+ T cells. HIV reservoir was quantified as total DNA (tDNA), unspliced RNA (usRNA), and intact DNA.

Results: After adjusting for nadir CD4+ count, timing of ART initiation, and genetic ancestry, we identified two host genes for which higher expression was significantly associated with smaller total DNA viral reservoir size, P3H3 and NBL1, both known tumor suppressor genes. We then identified 17 host genes for which lower expression was associated with higher residual transcription (HIV usRNA). These included novel associations with membrane channel (KCNJ2), inflammasome (NLRP3), TNFα, and innate immunity (TLR7) genes (FDR-adjusted q < 0.05). Gene set enrichment analyses further identified significant associations of HIV usRNA with TLR4/microbial translocation (q = 0.006), IL-1/IL-1α, IL-1β, IL-10, and TNFα protein associations achieving statistical significance (p < 0.05). Of note plasma IL-10 was also significantly inversely associated with HIV DNA (p = 0.016). HIV intact DNA was not associated with differential host gene expression, although this may have been due to a large number of undetectable values in our study.

Conclusion: To our knowledge, this is the largest cohort-based transcriptomic study of host genetic predictors of the HIV reservoir. Further studies are needed to validate these findings, ideally with dedicated functional genomic and intracellular protein assays using longitudinal samples to demonstrate causality of these observed associations. Our findings add important clinical and immunologic data to the limited host genomic HIV reservoir studies to date.

519 Taming the Viral Reservoir Over 3 Decades of Advancements in HIV Treatment

Irene González-Navarro1, Victor Ureña1, Cristina Gálvez2, Beatriz Mothe1, Lucía Balión1, María Salgado1, Javier Martínez-Picado1

1IroCasa Institute for AIDS Research, Badalona, Spain, 2Hospital Germans Trias i Pujol, Barcelona, Spain

Background: Since the advent of antiretroviral therapy (ART), HIV clinical management has steadily improved, not only because of the availability of more potent and safer drugs, but also due to the recommendations for universal test-and-treat for diagnosis, regardless of disease stage and CD4 count, and efforts to increase HIV diagnosis at its earliest stage after HIV acquisition. However, little is known about the effects of such improvements on the establishment of the latent HIV reservoir. Here, we characterize a group of people with HIV (PWH) on ART to determine the influence of multiple factors on the HIV reservoir evolution.

Methods: We analyzed the reservoir of 893 PWH treated and virologically suppressed for >3 years by measuring total HIV-DNA in PBMCs by ddPCR. Those with <50 HIV-DNA copies/10^6 PBMCs were classified as LoViReTs. 40 demographic, clinical, virologic, and immunologic variables were collected to explore their association with the LoViReTs status using the Random Forest machine learning algorithm, along with LOESS and logistic regression, or PCA.

Results: 180 (20%) of the 893 PWH were classified as LoViReTs. Minimum CD4 count, maximum viremia during clinical follow-up, shorter time from ART initiation to viral suppression, and longer time on an integrase strand transfer inhibitor (INSTI)-based regimen predicted LoViReTs status (classification error = 30%). The multiple logistic regression model estimation of the effect of these parameters was: minimum CD4 count OR = 1.52 (per 100 cells/μL), maximum viremia OR = 0.73 (log10 plasma HIV-RNA copies/mL), and time to suppression OR = 0.59 (years). We further analyzed how these factors vary based on ART start date. We observed a decrease in total HIV-DNA and a greater percentage of LoViReTs when ART was started after 2007. Indeed, time from treatment to suppression changed from 1.7 years in 1998 to <4 months in 2020. Since INSTIs were introduced in 2007, we performed a sub-analysis of the effect of INSTI-based regimens on HIV proviral levels, noting that individuals initiating ART with INSTIs had lower reservoir levels (p < 0.001) and shorter times to undetectable viremia (p = 2.2×10^-10).

Conclusion: ART guidelines constant improvement and the introduction of new generation drugs are related to the establishment of lower HIV reservoir levels in PWH and, therefore, to the increase in the LoViReTs phenotype among the individuals examined, which could facilitate the future success of medical strategies aimed at achieving a functional cure.

520 Vectored Delivery of Closer-to-Germline Antibodies Can Mediate Long-Term SHIV Suppression

Jose Martinez-Navio1, Sebastian P. Fuchs1, Desiree E. Mendes1, Claudia Muniz1, Paula G. Mondragon1, Rachel Zabizhin1, Eva Rakasz1, Guangping Gao1, Jeffrey Lifson1, Ronald C. Desrosiers1

1University of Miami, Miami, FL, USA, 2Wisconsin National Primate Research Center, Madison, WI, USA, 3University of Massachusetts, Worcester, MA, USA, 4AIDS and Cancer Virus Program, Frederick, MD, USA

Background: Delivery of potent broadly neutralizing antibodies by recombinant adeno-associated virus (AAV) vectors can strongly and durably
suppress ongoing viral replication after a single vector administration. We have successfully and durably suppressed viral replication in three SHIV-infected monkeys with this approach, including the "Miami monkey" with well over 7 years of plasma viremia below 15 copies/mL of SHIV RNA. However, the generation of anti-drug antibody responses (ADAs) against the AAV-delivered antibodies often limits the efficacy of this approach. Due to extensive affinity maturation, most potent broadly neutralizing antibodies exhibit unusually high levels of somatic hypermutation that can be seen as "non-self" by the recipient's immune system. To overcome this important challenge, delivery of less extensively mutated (i.e., closer-to-germline) antibodies was evaluated.

**Methods:** In a pilot experiment, four Indian-origin rhesus macaques were experimentally infected with SHIV-AD8. At week 14 post-infection, and with plasma viremias ranging from 5,100 to 130,000 copies/mL of SHIV RNA, all four monkeys received AAV vectors expressing anti-HIV antibodies DH270, PCIN63 and DH511. These antibodies are naturally closer-to-germline than those we had previously used.

**Results:** Three of the four macaques showed high levels (20-320 µg/mL) of two AAV-delivered antibodies through 70 weeks of follow up measurements. Sustained viral load suppression was achieved in two of these three monkeys. Only transient effects on viral load levels were observed in the third monkey suggesting viral escape mutations; sequencing to confirm escape is currently in progress. The fourth monkey showed low levels of AAV-delivered antibodies due to ADAs and little or no viremic suppression.

**Conclusion:** Our results indicate that potent broadly neutralizing antibodies which are closer-to-germline can mediate long-term virologic suppression following gene therapy with AAV vectors with potentially lower incidence of ADA responses, but also highlight the difficulties associated with achieving long-term, antibody-mediated suppression.

### 521 Ruxolitinib-Mediated HIV-1 Reservoir Decay in A5336 Phase Ila Trial

**Background:** Ruxolitinib is FDA approved for myelofibrosis, polycythemia vera, atopic dermatitis and chronic graft-versus-host disease. We evaluated ruxolitinib's impact on the peripheral HIV-1 reservoir and key immunomodulatory events driving HIV-1 persistence in people with HIV-1 (PWH) in an AIDS Clinical Trial (ACTG) sponsored open-label randomized Phase 2a multi-site trial (n=60).

**Methods:** Inclusion criteria: ≥18 age ≤75, background ART regimen (NNRTI or INSTI without cobicistat for ≥2 years), continuously virologically suppressed, CD4+ T-cell count >350 cells/mm³; no significant medical condition besides HIV or hypertension. Participants were randomized to ruxolitinib 10 mg bid plus ART (n=40) or ART alone (n=20) from week 0 through 5. Both groups were observed through week 12. Cellular markers, integrated DNA, and IPDA were measured on peripheral blood from weeks 0, 5, and 12. A sequential series of Mann Whitney U tests were performed to understand how biomarker changes across weeks impact reservoir decay. We evaluated 1) biomarkers within the ruxolitinib-treated (RUX) group which were altered from baseline, 2) which markers determined in round 1 were differential versus control (CNT) group, and 3) which of these were associated with participants who experienced reservoir growth or decay independent of treatment. Significance was further confirmed through Benjamini-Hochberg testing to minimize the false discovery rate (15%).

**Results:** Integrated DNA and IPDA reservoir measurements were highly correlated to one another throughout trial. HIV-1 reservoir significantly decayed in the RUX group by week 12 versus CNT (p=0.0471). Cellular markers altered by ruxolitinib, possessing a different expression profile from CNT, and associated with reservoir decay were determined: pSSTAT5+ monocytes in low baseline reservoir and total RUX groups at week 12, pSSTAT3+ monocytes in low baseline reservoir at week 12, BCL-2/XIAP+ CD4+ T cells in the total RUX group at week 5, CD27+ CD8+ T cells in the total RUX group at week 5, and CD25+ CD8+ TTM cells in the total RUX group at week 5.

**Conclusion:** Ruxolitinib decays the HIV-1 reservoir and resets immune balance in PWH on ART. Based on observed reservoir decay (week 5 to 12), our model predicts a 99.99% decay in 2.83 years, should rate of decay remain constant. These data are foundational for future human trials with Jak 1/2 inhibitors such as ruxolitinib towards HIV-1 elimination.

### 522 Short-Term BCL-2 Inhibition at ART Initiation Transiently Reduces SIV Reservoir in Rhesus Macaques

**Background:** In spite of the success of antiretroviral therapy ART in suppressing HIV infection, effective strategies to limit the establishment and maintenance of the HIV reservoir are needed. Recent data highlighted BCL-2 as a key regulator for the establishment, persistence, and expansion of the CD4+ T cell reservoir, protecting infected cells from virus or CD8 T cell-mediated cell death. Thus, there is a strong rationale behind promoting apoptosis of latently HIV-infected cells via BCL-2 inhibition. Herein, we evaluate in SIV-infected, ART-treated rhesus macaques (RMs) the effect of the BCL-2 inhibitor venetoclax, alone or combined with a latency-reversing strategy (CD8a cell depletion), on cellular dynamics and the size of the SIV reservoir.

**Methods:** 12 RMs were intraocularly infected with barcoded SIVmac239 and initiated ART 2 weeks post-infection. RMs were distributed into 3 arms: 4 RMs received only ART, 4 RMs received 10 daily doses of venetoclax as a subcutaneous injection starting at ART initiation; and 4 RMs received venetoclax in conjunction with a single dose of the anti-CD8a depleting antibody MT807R1 at ART initiation. Animals were followed until day 70 post-infection.

**Results:** Venetoclax and venetoclax plus MT807R1 treated RMs had a slower viral plasma load decay rate than control RMs, with prolonged on-ART viremia. In blood, venetoclax-depleted CD4+ T cells, including their central and effector memory subsets. This was accompanied by decreased total SIV-DNA in peripheral blood mononuclear cells and intact SIV-DNA in sorted CD4+ T cells, consistent with a preferential loss of infected cells following venetoclax administration. Furthermore, and despite the slower plasma viral load decay, venetoclax-treated animals showed a faster intact SIV-DNA decay rate, consistent with killing of SIV-DNA harboring cells. This effect was specific to the treatment and not sustained after the interruption of Venetoclax.

**Conclusion:** This study shows that BCL-2 inhibition at ART initiation can delay the establishment of latency and transiently deplete infected cells by promoting the elimination of SIV-infected circulating CD4+ T cells. The results support the concept that BCL-2 expression favors the survival of infected cells and represents a potential therapeutic target. Further studies are needed to explore the effect of BCL-2 inhibition during long-term ART and prolonged exposure to BCL-2 inhibitors.

### 523 CD4-Targeted mRNA Delivery of Tat Reverses HIV-1 Latency

**Background:** Interventions that reverse viral latency are needed for an HIV cure. The viral protein Tat drives HIV expression but is toxic and difficult to deliver in a targeted manner. We tested whether CD4-targeted delivery of Tat encoding mRNA could safely reverse latency.

**Methods:** mRNA encoding wildtype and nontoxic Subtype B and D Tat variants were made and transfected into TZM-bl s to assess transactivation. Five J-Lat clones were treated with Tat mRNA-LNPs or small molecule agents (romidepsin, prostratin, pabinostat, or AZD5582) and evaluated using flow cytometry, live cell imaging, or single cell multiomic (RNA+ATAC) profiling. Ibalizumab (anti-CD4) was conjugated to mRNA-LNPs to generate CD4-targeted mRNA-LNPs. Clones were treated with Tat mRNA-LNPs or small molecule agents (romidepsin, prostratin, pabinostat, or AZD5582) and evaluated using flow cytometry, live cell imaging, or single cell multiomic (RNA+ATAC) profiling. Ibalizumab (anti-CD4) was conjugated to mRNA-LNPs to generate CD4-targeted mRNA-LNPs. Primary CD4 T cells were treated with CD4-targeted Tat or GFP mRNA-LNPs to assess mRNA expression, toxicity, and activation. PBMCs from people living with HIV (PLWH) on ART were treated with CD4-targeted Tat mRNA-LNPs and assayed for p24 expression.

**Results:** A nontoxic Tat variant demonstrated similar transactivation activity when compared to wildtype variants and drove HIV expression in neighboring, nontransfected cells, indicating secretion of active Tat. Tat mRNA transfection of J-Lat 10.6 resulted in dose-dependent viral reactivation. Multiomic analysis of treated J-Lat 10.6 revealed significant changes in expression of four genes (log₂ fold change) > 0.25, p < 0.05: HIV-1 (0.37 log₂ fold increase),
MTNR2L2 (0.33 log₂-fold decrease), RPS10 (0.31 log₂-fold decrease), and SNHG25 (0.26 log₂-fold decrease). Minimal changes to ATAC accessibility of the proviral genome 2-3 days post-treatment were seen despite GFP signal in ~60% of cells. All J-Lat clones reactivated, albeit heterogeneously, upon treatment with Tat mRNA-LNPs or Romdepsin, but less so with other agents. Ibalizumab-targeting of Tat or GFP mRNA-LNPs resulted in efficient mRNA delivery and expression in rhesus and human CD4 T cells, with no observed cytotoxicity (Live/Dead, Annexin V), change in activation markers (CD69, CD25, CD38, HLA-DR), or widespread changes to gene expression in bulk RNAseq. CD4-targeted Tat mRNA-LNP treatment of PBMCs from three PLWH with inducible reservoirs resulted in p24 expression in both cellular pellet (0.05-0.5 pg/ml) and supernatant (0.15-0.2 pg/ml).

**Conclusion:** CD4-targeted Tat mRNA-LNPs selectively drive viral gene expression without cytotoxicity. These findings show that viral transcription programs, such as latency, can be modified using cell-targeted mRNA gene therapy.

### 524 Potent HIV Latency Reversal by Lipid Nanoparticles Encapsulating HIV Tat mRNA

**Bridge M. Fisher, Paula M. Cevaia, Stanislav Kan, Abdalla Ali, Abigail Tan, Rory Shepherd, Youy Kim, Marvin Holz, Damian Purcell, Frank Caruso, Sharon R. Lewin, Michael Roche**

**University of Melbourne, Melbourne, Australia**

**Background:** One approach to eliminating the HIV reservoir is to upregulate proviral transcription and protein production, to induce the clearance of latently infected cells. To date, latency reversal agents (LRAs) have demonstrated poor efficacy in reversing latency and exhibited off-target effects. This is likely due to targeting host pathways and transcription factors. Here we developed lipid nanoparticles (LNPs) encapsulating mRNA encoding for the HIV Trans-activator of Transcription (Tat) protein, and characterised their ability to reverse latency in a potent, HIV-specific manner.

**Methods:** LNPs encapsulating mRNA encoding either Tat (Tat-LNP) or mCherry reporter protein (mCherry-LNP) were formulated through microfluidic mixing. Transfection efficiency was assessed in non-stimulated CD4+ T cells from HIV-negative donors. HIV latency reversal was assessed in the latently infected J-Lat A2 cell line by measuring GFP induction, and ex vivo in CD4+ T cells isolated from people living with HIV (PLWH) on suppressive antiretroviral therapy (ART). Using digital RT-PCR measuring various HIV transcripts. Cellular activation and viability were characterised ex vivo by assessing CD69, CD25 and HLA/DR expression by flow cytometry.

**Results:** mCherry-LNPs potently transfected non-stimulated CD4+ T cells (75±3.6% mean±SEM mCherry+ cells). Tat-LNPs induced HIV reactivation in J-Lat A2 cells, with a fold increase of 68.2±10.3 (mean±SEM; EC50 = 3.9 ng/ml) relative to untreated. In ex vivo CD4+ T cells from PLWH on suppressive ART the proviral genome 2-3 days post-treatment were seen despite GFP signal in ~60% of cells. All J-Lat clones reactivated, albeit heterogeneously, upon treatment with Tat mRNA-LNPs or Romdepsin, but less so with other agents. Ibalizumab-targeting of Tat or GFP mRNA-LNPs resulted in efficient mRNA delivery and expression in rhesus and human CD4 T cells, with no observed cytotoxicity (Live/Dead, Annexin V), change in activation markers (CD69, CD25, CD38, HLA-DR), or widespread changes to gene expression in bulk RNAseq. CD4-targeted Tat mRNA-LNP treatment of PBMCs from three PLWH with inducible reservoirs resulted in p24 expression in both cellular pellet (0.05-0.5 pg/ml) and supernatant (0.15-0.2 pg/ml).

**Conclusion:** CD4-targeted Tat mRNA-LNPs selectively drive viral gene expression without cytotoxicity. These findings show that viral transcription programs, such as latency, can be modified using cell-targeted mRNA gene therapy.

### 526 Non-Neutralizing Antibody Therapeutics to Eliminate HIV-Infected Cells In Vitro and In Vivo via ADC

Jonathan Richard, Dung N. Nguyen, William D. Tolbert, Derek Yang, Seung Tae Kim, Marco A. Diaz-Salinas, Jyoth K. Rajasekhar, Li Zhu, Catherine Bourassa, Halima Medjahed, Priti Kumar, James B. Murno, Amos B. Smith, Andrés Fizzzi, Marzena Pazgier

1Centre de Recherche du CHUM, Montreal, Canada, 2Uniformed Services Universities of the Health Sciences, Bethesda, MD, USA, 3University of Pennsylvania, Philadelphia, PA, USA, 4University of Massachusetts, Worcester, MA, USA, 5Yale University, New Haven, CT, USA

**Background:** In HIV-1 infection, a significant fraction of antibodies (Abs) is induced to epitopes occluded in the Env trimmer at the surface of infected cells and viral particles that are exposed only upon Env interaction with CD4 (CD4-induced or CD4). Most CD4 Abs are weakly or non-neutralizing (mAbs) but their FcR interactions, including antibody-dependent cytotoxicity (ADCC), are usually potent, but strictly dependent on epitope exposure. Studies have shown that HIV-1 evolved to restrict Env's contact with CD4 to avoid ADCC induced by CD4i mAbs. Interestingly, some CD4i epitopes, including the co-receptor binding site (CoRBS) or gp120 Cluster A, represent the most conserved Env regions, suggesting their great potential as targets for AB-based approaches.

**Methods:** We developed CoRBS and Cluster A Abs into Ab-CD4 hybrids or Ab-CD4 mimetic (mc) conjugates in which an Ab (e.g. 17b or A32) is linked via a (G4S)6(G4T)2-linker, respectively. Ab therapeutics were evaluated via a (PEG)23-linker, respectively. Ab therapeutics were evaluated for binding and ADCC mediated elimination of HIV-1-infected primary CD4+ T cells, and in vivo for their ability to eliminate infected cells in HIV-1JRFL-infected humanized mice supporting NK cell functions.

**Results:** In vitro and vivo setting, the AB-CD4 hybrids of both specificities efficiently eliminated cells infected with HIV-1JRFL with potencies outperforming the best-in-class bnAbs. In addition, these AB-CD4s were also able to synergize with mAbs present in HIV+ plasma, further enhancing their ADCC activity. Our experiments utilizing single molecule FRET (smFRET) imaging confirmed that the activity of the AB-CD4s directly results from their ability to stabilize State 2A, an Env conformation known to be ADCC vulnerable. Furthermore, results in humanized mice showed that A32-CD4 can control HIV-1 replication and substantially reduce the level of integrated HIV DNA in an FcR-dependent manner. Finally, our data also indicates that the CD4 moiety in an Ab-CD4 hybrid can be replaced by a small molecule CD4 mimetic. Our preliminary data confirm in vitro activity to eliminate HIV-1infected cells with an Ab-CIF-III-288 conjugate utilizing an Ab of the CoRBS specificity.
527 A New CD4mc Enables Fc-Mediated Reservoir Reduction for Durable Post-ART HIV-1 Control in Hu-Mice

Li Zhu1, Sri Lakshmi T. Boodapati1, Jonathan Richard2, Christopher J. Fritsch1, Derek Yang1, Hongil Kim1, Yaping Sun1, Lorie Marchitto1, Guillaume Beaudoin-Bussieres1, Debashree Chatterjee1, Catherine Bourassa1, Joseph Sodroski1, Amos B. Smith III1, Andrés Finzi1, Priti Kumar1

1Yale University, New Haven, CT, USA, 2Centre de Recherche du CHUM, Montreal, Canada, 3University of Pennsylvania, Philadelphia, PA, USA, 4Dana–Farber Cancer Institute, Boston, MA, USA

Background: Persistently-infected HIV-1-positive cells are heterogeneous, rare, and inherently difficult to eliminate; shock-and-kill approaches have thus far proven inefficient in measurably reducing infected cell frequencies in clinical trials. CD4-mimetic compounds (CD4mcs) sensitize HIV-1-infected cells to antibody-dependent cellular cytotoxicity (ADCC) mediated by CD4-induced (CD4i) non-neutralizing antibodies (nAbs) that are frequently found in plasma of people living with HIV (PLWH) (Richard et al., PNAS 2015, PMID: 25941367; Rajashekar, new richard et al., Cell Host Microbe 2021, PMID: 34019804). The development of a new indoline CD4mc, CFI-III-288, with improved potency in neutralization and ADCC (Fritsch et al., PNAS 2023, PMID: 36961924), prompted us to investigate the impact of treatment, administrated at ART initiation or ART cessation, on viral tissue reservoirs and post-ART virus rebound dynamics in humanized mice (hu-mice) that support antibody effector function.

Methods: NSG-Tg(IL7) hu-mice were treated with CFI-III-288 in combination with CD4i A32 (anti-cluster A) and 17b (anti-coreceptor binding site) nAbs either at ART initiation or at ART withdrawal. Post-ART plasma viremia and tissue viral reservoir size were analyzed by longitudinal measurements of HIV-1 RNA and DNA respectively. We also analyzed the contributions of natural killer (NK) and CD8 T cells to the observed effects in in vivo cell-depletion studies.

Results: Striking differences in HIV-1 rebound dynamics were observed depending on the timing of CFI-III-288/CD4i nAb treatment. While treatment at ART cessation resulted in a significant delay of viral rebound (up to 1 month), treatment at ART initiation had an even greater effect, delaying viral rebound by more than twice as long at treatment at ART cessation. Remarkably, durable control of plasma viral loads (in spite of transient viremic blips) with a highly significant reduction of the viral reservoir led to ART-free viral remission in a subset of mice treated during the viremic phase of infection, concomitant with initiation of ART. While the outcome was primarily mediated by NK cells supporting the major contribution of ADCC effects, a role for CD8+ T cells in the continued suppression of viral loads and control of viral rebound was suggested from the results of in vivo cell-depletion experiments.

Conclusion: The overall results indicate that CD4mc have therapeutic potential in the presence of anti-Env CD4i antibodies, especially when administered at early stage.

528 Combination CAR T-Cell Therapy Restricts HIV Escape and Durably Suppresses HIV Replication In Vivo

Federica Severi1, Alexandra Criswell2, Dalia Becrow2, Tyler Yang2, Francesco Pennino3, Reyes Acosta4, Daniel T. Claiborne4

Wistar Institute, Philadelphia, PA, USA

Background: In most cases, the cellular immune response is unable to durably control HIV replication. Chimeric antigen receptor (CAR) T cell therapy provides an avenue to engineer a more potent T cell response. Broadly neutralizing antibodies (bNAb) targeting conserved regions of HIV Envelope may further facilitate the creation of a CAR T cell therapy effective against the majority of HIV isolates.

Methods: A panel of 4-1BB costimulated CAR T cells expressing 18 distinct bNAb-based CARs were generated against all well-characterized epitopes on HIV Env: V1/V2 apex, V3-glycan supersite, CD4 binding site, gp120-gp41 interface, fusion peptide, silent face, and MPER. CAR T products manufactured from primary human T cells were screened for potency via degranulation when cocultured with Env-expressing K562 (K.Env) cells, as well as K.Env killing assays and HIV suppression assays in the GKR25 cell line using the Incyte SXS live-cell imaging system. To map escape pathways and determine CAR efficacy, donor-matched CAR T products were adaptively transferred into HIV-infected, BLT humanized mice. CAR expansion and HIV viral loads were monitored weekly in the blood by flow cytometry and qRT-PCR, and HIV env sequences were amplified from plasma at >6 weeks post infection using nested PCR and deep sequenced to identify putative escape mutations.

Results: Potency hierarchy, as measured by degranulation potential, K.Env killing kinetics, and effective suppression of virus replication in vitro was heterogeneous among bNAb CAR constructs, but clustered between epitope specificities. Notably, Envs epitopes most distal (V1/V2 apex, V3-glycan supersite) from the plasma membrane were associated with greater killing potency. Deep sequencing of HIV env derived from bNAb CAR T-treated HIV-infected humanized mice identified bNAb CARs with overlapping versus orthogonal associated escape pathways. This data guided the design of bNAb CAR combinations hypothesized to restrict escape. Using this approach, we demonstrate that a triple combination of V1/V2 apex, V3-glycan supersite, and CD4 binding site-directed CARs were able to durably suppress acute viremia to undetectable levels in humanized mice.

Conclusion: Distinct in vitro assays identified bNAb CARs with superior potency (PGT128, PGM1400). HIV escape from individual bNAb CARs can be restricted when CARs associated with orthogonal escape pathways are combined, and restriction of HIV escape leads to durable suppression of HIV replication in vivo.

529 Vaccination Combined With PD-1 Blockade Provides Sustained SIV Suppression in Mamu-A01+(+) Macaques

Bhruag Yagnik1, Sheikh A. Rahmam1, Sallaja Gangadhara1, Shan Liang2, Gordon Freeman1, Rafi Ahmed1, Rama R. Amara1

1Emory University, Atlanta, GA, USA, 2Harvard Medical School, Boston, MA, USA, 3Emory Vaccine Center, Atlanta, GA, USA

Background: Dysfunctional T cells and persistent viral reservoir under anti-retroviral therapy (ART) are the two major challenges to HIV cure. Towards this, here we evaluated therapeutic potential of intramuscular (IM) or intravenous (IV) vaccination combined with a latency reversal agent (LRA) and PD-1 blockade in SIV infected macaques.

Methods: A total of 28 RMs were infected with SIVmac251, placed on daily ART at 2 weeks post infection (wpi) and divided into three groups. Two groups received two DNA/SIV vaccinations (34, 40 wpi; ID) followed by two modified vaccinia Ankara (MVA)/SIV vaccines (46, 66 wpi) via either IM (MVA-IM, n=14) or IV (MVA-IV, n=7), and the third group did not receive any vaccination (ART, n=7). All RMs received five weekly IV infusions of AZD5582 from weeks 52 to 56 under ART as an LRA after the 1st MVA. On the day of ATI (75 wpi), 7 RMs from MVA-IM group received six infusions of a primatized anti-PD-1 antibody (10mg/kg body wt.) at three-week intervals (MVA-IM+PD-1). Viral rebound kinetics were studied for up to 32 weeks post ATI. In-depth immunological, virological, and reservoir analyses were performed throughout the study.

Results: Both IM and IV routes of therapeutic vaccination generated broad and polyfunctional SIV-specific CD4+ and CD8+ T cells in blood and lymph nodes. Surprisingly, administration of AZD5582 under ART led to a significant loss of vaccine-induced effector CD8 T cells but were restored to high frequencies following the 2nd MVA. Post ATI, all animals showed a strong viral rebound. The ART, MVA-IM and MVA-IV animals, irrespective of Mamu-A01 status, did not control rebounding viremia. However, anti-PD-1 Ab treated Mamu-A01(+) animals (n=4) showed a profound suppression (below 60 copies with small blips under 1000 copies/ml) of reemerging viremia up to 32 weeks post ATI. The PD-1 blockade also induced high frequencies of polyfunctional SIV-specific cytolytic (granzyme B+, perforin+) CD8 T cells in the T-cell zone and B cell follicles of LNs, which were associated with sustained viral control.

Conclusion: A combination of vaccination during ART and PD-1 blockade post ATI can achieve a sustained functional cure for SIV in the presence of a potent anti-viral CD8 T cell response. These results also highlight the need for optimization of AZD5582 treatments in conjunction with vaccination to prevent the loss of vaccine-induced CD8 T cells.
530 The Impact of HIV Cure-Related Study Drugs and Treatment Interruption on Viral Resurrection Rates
Ming J. Lee1, Miles Eason1, Antonella Castagna1, Laura Galli1, Marie-Angeilique De Schenderey1, James L. Riley1, Pablo Taboas1, Jesper D. Gunt1, Ole S. Segard2, Eric Florence2, Eugene Kroon3, Mark S. de Souza3, Beatriz Mothe4, Marina Caskey4, Sarah Kid4, Imperial College London, London, United Kingdom, San Raffaele Vita-Salute University, Milan, Italy, Sam Raffaele Institute, Milan, Italy, Ghent University, Ghent, Belgium, University of Pennsylvania, Philadelphia, PA, USA, Aarhus University Hospital, Aarhus, Denmark, Institute of Tropical Medicine, Antwerp, Belgium, SEARCI, Bangkok, Thailand, Infectiome Institute for AIDS Research, Badalona, Spain, The Rockefeller University, New York, NY, USA

Background: To assess the effectiveness of novel HIV curative strategies, "cure" trials require periods of closely monitored antiretroviral therapy (ART) analytical treatment interruptions (ATI). We performed a systematic review and meta-analysis to identify the impact of ATI with or without novel therapeutics in cure-related studies on the time to viral resurrection following ART restart.

Methods: Medline and Embase databases were searched for human studies involving ATIs from January 2015 to March 2023. The primary outcome was proportion of participants who experienced viral resurrection (plasma HIV viral load (VL) <50 copies/mL) by 12 weeks post-ATI, stratified by receipt of interventional drug with ATI (IA) or ATI-only groups. A random-effects proportional meta-analysis and multivariable Cox proportional hazards analysis were performed using R.

Results: Of 1049 studies screened, 12 were relevant with available data and included in the analysis (n=180 participants). 96% of participants achieved viral resurrection within 12 weeks (95% confidence interval (CI): 83% - 99%), with no difference between ATI-only and IA subgroups (97% vs 96%, p=0.079) (Figure 1). In the adjusted time-to-event analysis, age (adjusted Hazard Ratio (aHR) 0.97, 95% CI 0.96 - 0.99, p<0.001), greater VL at ART restart (aHR 0.47, 95%CI (0.38 - 0.59, p<0.001), use of protease inhibitors (aHR 0.34, p=0.19 - 0.73, p=0.004), duration of ATI (aHR 0.96, 95%CI 0.94 - 0.98), and longer intervals between HIV VL monitoring (aHR 0.69, 95%CI 0.59 - 0.74, p<0.001) were associated with a decreased likelihood of achieving resursion after restarting ART, but not receipt of trial interventions (HR 0.89, 95%CI 0.57 - 1.40, p=0.612).

Conclusion: 96% of study participants who underwent ATIs achieved viral resursion within 12 weeks after restarting ART and this outcome did not differ with or without the receipt of any interventional drugs. When designing studies involving ATIs, time to viral resurrection after restarting ART should be regularly monitored and reported, to assess the impact and safety of specific trial interventions in ATI studies.

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532 Treatment With AZD5582 + hetIL-15 Disrupts the Reservoir Establishment in SIV-Infected Macaques
Maura Statzu1, Cristina Micali1, Tomas Raul Wiche Salinas1, Catherine Garley1, Brandon Healy2, Diane G. Carnathan1, Brandon Keeler1, Jeffrey Lisson1, Gregory M. Laird1, David M. Mangels1, Mirko Piai1, Guido Silvestri1

1Emory University, Atlanta, GA, USA, 2Friederici National Laboratory for Cancer Research, Frederick, MD, USA, 3Accelware Diagnostics, Baltimore, MD, USA, 4University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: HIV infection cannot be cured despite suppressive antiretroviral therapy (ART) due to a persistent reservoir of latently-infected memory CD4 T cells harboring replication competent virus. Here, we tested the SMCam/iAP antagonist AZD5582 in combination with the heterodimeric interleukin-15 (hetIL-15), the native form of the cytokine that activates and expands cytotoxic T and NK cells, as a novel approach to prevent the establishment of the virus reservoir during the early stages of infection and/or ART.

Methods: 31 rhesus macaques (RMs) were infected with genetically barcoded SIVmac239M and started ART at 2-weeks p.i. 9 RMs received hetIL-15 via sq injections of escalating dose of 10-40 ug/kg (weekly for 10 weeks) starting 3 days before ART initiation; 9 RMs received iv AZD5582 at 0.1 mg/kg weekly for 10 weeks starting at ART initiation; 9 RMs received combination of hetIL-15 and AZD5582 over the same experimental phase as outlined above. Animals served as treatment-naïve, ART-only controls. Plasma viral loads were measured longitudinally for 16 weeks and the reservoir size was estimated in PBMC and lymph nodes (LN) by IPDA. CD4 T cells, CD8 T cells, and NK cells phenotypes were characterized by flow cytometry.

Results: Treatment with AZD5582, alone or in combination with hetIL-15, resulted in slower decline of plasma viremia after ART initiation, and SIV viral load was significantly higher at day 14–15–17–21–25–49 and 70p.i. in AZD5582 treated RMs, indicating that treatment with the SMCam associated with a longer lifespan of the productively infected cells. Levels of peripheral and LN CD4 T cell intact proviral SIV DNA declined in all the groups over the treatment course. The frequency of IPDA + CD4 T cells tended to be lower in the animals receiving the SMCam, alone or in combination with hetIL-15, with a statistically significant difference at day 25 p.i in both PBMC and LN. Interestingly, levels of granzyme B at day 25 p.i were higher in LN CD8 T cells of the treated animals compared to the controls.

Conclusion: Altogether, these findings suggest that treatment with AZD5582, alone or in combination with hetIL-15, may reduce the size of virus reservoir when administered at the time of ART initiation during acute SIV infection, thus suggesting a disruptive effect on the reservoir establishment. These data are consistent with previous work on the latency reversing activity of AZD5582 and provides rationale for further exploring this compound as a curative agent for HIV infection.

After HIV infection through qPCR, western, ELISA, IHC and RNAseq. BLT hu-mice were infected with transmitted/founder (T/F) virus HIV1usu and following 4 to 5 weeks of viremia were suppressed on cART (FTC+TDF+RAL). Infusions of anti-IFNa/b-specific or IgG control antibodies were administered to the HIV fully suppressed animals intraperitoneally over three independent studies. Viral measures (plasma viral load, proviral DNA load), leukocyte subset activation, and HIV-antigen specific T cell function were measured by flow cytometry. Analysis was done with Prism software.

Results: Neutralizing anti-IFNa or anti-IFNb-specific antibodies are capable of specifically blocking IFNa or IP18 signaling in vitro and in vivo. We confirmed IFNa and IFNb expression in BLT humanized mice, and the increase in ISGs expression after HIV infection. We found the infusions of anti-IFNa-beta but not IFNa or IgG control can repeatedly elicit repeated low level viremic blips under ART. HIV proviral,total DNA size were increased right after the IFNb-specific antibody treatment supporting viral expression. HIV gag-specific CD8+ T cell frequency (IFN-gamma and CD107a) was also increased on ART after anti-IFNb-specific antibody treatment consistent with HIV antigen expression. However, no change in viral rebound after stopping ART was detected between groups.

Conclusion: Blockade of IFN-beta signaling can reactivate HIV reservoir and stimulate HIV-specific T cells under ART in HIV infected BLT humanized mice. The potential use of anti-IFNa strategies together with direct anti-HIV “kill” or clearance strategies should be further investigated.

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531 Blockade of IFN-beta Reactivates HIV Reservoir in ART-Suppressed HIV-Infected BLT Humanized Mice
Zhe Yuan1, Emmanuel Papavassiliou2, Guorui Zu1, Lily Lu1, Matthew Fairs1, Samuel Keller1, Luca Sardo1, Guoxin Wu1, Joel Casset1, Joseph Salvo1, Pau Zuck1, Bonnie Howell1, Luis J. Monnat1

1Wistar Institute, Philadelphia, PA, USA, 2Merck & Co, Inc, Kenilworth, NJ, USA

Background: Despite the efficient suppression of HIV-1 replication can be achieved with combined antiretroviral therapy (cART), viral latency and low levels of type I interferon (IFN-1) signaling persist during chronic infection. This sustained signaling may promote T cell exhaustion and foster viral persistence. Using cloned human neutralizing antibodies specifically against IFNa or IFNb, here we test the effects of blocking IFNa or IFNb in ART suppressed BLT humanized mice to determine the role each Type I IFN in suppression.

Methods: Antibodies used for infusions against IFNa or IFNb were characterized by ELISA, STAT-1 phosphorylation, dimerization assay, and western blot to confirm specificity. Tissues and plasma from BLT humanized mice were used to measure IFNa and IFNb expression as well as ISGs expression after HIV infection through qPCR, western, ELISA, IHC and RNAseq. BLT hu-mice were infected with transmitted/founder (T/F) virus HIV1usu and following 4 to 5 weeks of viremia were suppressed on cART (FTC+TDF+RAL). Infusions of anti-IFNa/b-specific or IgG control antibodies were administered to the HIV fully suppressed animals intraperitoneally over three independent studies. Viral measures (plasma viral load, proviral DNA load), leukocyte subset activation, and HIV-antigen specific T cell function were measured by flow cytometry. Analysis was done with Prism software.

Results: Neutralizing anti-IFNa or anti-IFNb-specific antibodies are capable of specifically blocking IFNa or IFNb signaling in vitro and in vivo. We confirmed IFNa and IFNb expression in BLT humanized mice, and the increase in ISGs expression after HIV infection. We found the infusions of anti-IFNa-beta but not IFNa or IgG control can repeatedly elicit repeated low level viremic blips under ART. HIV proviral,total DNA size were increased right after the IFNb-specific antibody treatment supporting viral expression. HIV gag-specific CD8+ T cell frequency (IFN-gamma and CD107a) was also increased on ART after anti-IFNb-specific antibody treatment consistent with HIV antigen expression. However, no change in viral rebound after stopping ART was detected between groups.

Conclusion: Blockade of IFN-beta signaling can reactivate HIV reservoir and stimulate HIV-specific T cells under ART in HIV infected BLT humanized mice. The potential use of anti-IFNa strategies together with direct anti-HIV “kill” or clearance strategies should be further investigated.

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533 Drug-Controlled Anti-PD-1 CAR T-Cells to Target the Replication-Competent Reservoir in Tfh Cells
Karsten Eichholz1, Yoshinori Fukazawa1, Christopher W. Peterson1, Francois Haeuserle1, Benjamin Varco-Merth1, Sandra Dross2, Hsuan Sun Park3, Carayln S. Labriola4, Michael Afxeldom5, Jeremy Smalley6, Hans-Peter Kiem7, Louis Picker8, Aman A. Okoye9, Corey Larry9
1Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 2Oregon Health and Sciences University, Portland, OR, USA, 3Fred Hutchinson Cancer Center, Seattle, WA, USA, 4University of Washington, Seattle, WA, USA

Background: Programmed cell death protein 1 (PD-1) is an immune checkpoint marker expressed on memory T cells and enriched in latently infected CD4+ T cells and T follicular helper cells (Tfh) containing replication-competent human immunodeficiency virus 1 (HIV) proviruses in people with HIV on antiretroviral therapy (ART). We recently tested a novel anti-PD-1 chimeric antigen receptor (CAR) T cell approach in vivo in simian immunodeficiency virus (SIV) mac239-infected and SIV-naïve rhesus macaques (RM) to assess the impact of PD-1 depletion on viral reservoirs and rebound dynamics. We found that anti-PD-1 CAR T cells expanded efficiently and rapidly eradicated all Tfh cells from the germinal centers with concomitant depletion of detectable SIV RNA from this sanctuary site. This occurred even before release from ART. The anti-PD-1 CAR T cells persisted for up to 100 days concomitant with the depletion of PD-1+ memory T cells in blood and tissues, resulting in off target immune depletion and a marked increase in SIV replication in extrafollicular portions of lymph nodes, a 2-log higher plasma viremia relative to controls and accelerated disease progression, associated with the acute depletion of CD8+ memory T cells after CAR T expansion. The rapid depletion of Tfh sanctuary site in GC while on ART offered the potential to an important advance in the quest for HIV cure if we could develop a second-generation product that had less off target long term effects.

Methods: Towards these ends, we integrated a Hepatitis C virus-derived non-structural protein 3 (NS3)-based ON switch into the intracellular domain of our anti-PD-1 CAR (NS3) T cell platform that can be controlled exogenously by administration of a NS3-specific protease inhibitor Grazoprevir (GZV).

Results: Mechanistically, the NS3 domain undergoes autocleavage in absence of GZV, thus abrogating any CAR signaling and CAR T cell function. In vitro, primary T cells expressing the anti-PD-1 CAR NS3 kill PD-1+ cells and secret IFNγ in presence of GZV in a dose-dependent manner indicating that the NS3 domain functions as an ON switch.

Conclusion: Combined, these data indicate that a drug-controlled anti-PD-1 CAR NS3 is highly functional and can be controlled exogenously through administration or withdrawal of GZV. These data warrant further investigation of drug-controlled anti-PD-1 CAR T cells in ART-treated SIVmac239-infected rhesus macaques to transiently deplete Tfh cells to determine their contribution to the latent reservoir.

534 Refractoriness to SIV Reinfection Is Induced by Anti-IL-10/PD-1 Therapy in Rhesus Macaques
Susan P. Ribeiro1, Felipen Ten Caten1, Khader Ghneim2, Zachary Strongin2, Kevin Nguyen3, Justin L. Harper4, Robert Balderas3, Luca Micci4, Jeffrey Lifson5, Daria Hazuda3, Daniel Gorman1, Bonnie Howell1, Mirko Pajardini2, Rafic P. Sekaly1
1Emory University, Atlanta, GA, USA, 2Merck & Co, Inc, Rahway, NJ, USA, 3BD Biosciences, Franklin Lakes, NJ, USA, 4Frederick National Laboratory for Cancer Research, Frederick, MD, USA, 5Generate Biomedicines, Somerville, MA, USA, 6Emory National Primate Research Center, Atlanta, GA, USA

Background: Programmed cell death protein 1 (PD-1) is an immune checkpoint molecule expressed on memory T cells and enriched in latently-infected CD4+ T cells from the germinal centers with concomitant depletion of detectable SIV RNA from this sanctuary site. This occurred even before release from ART. The anti-PD-1 CAR T cells persisted for up to 100 days concomitant with the depletion of PD-1+ memory T cells in blood and tissues, resulting in off target immune depletion and a marked increase in SIV replication in extrafollicular portions of lymph nodes, a 2-log higher plasma viremia relative to controls and accelerated disease progression, associated with the acute depletion of CD8+ memory T cells after CAR T expansion. The rapid depletion of Tfh sanctuary site in GC while on ART offered the potential to an important advance in the quest for HIV cure if we could develop a second-generation product that had less off target long term effects.

Methods: Towards these ends, we integrated a Hepatitis C virus-derived non-structural protein 3 (NS3)-based ON switch into the intracellular domain of our anti-PD-1 CAR (NS3) T cell platform that can be controlled exogenously by administration of a NS3-specific protease inhibitor Grazoprevir (GZV).

Results: Mechanistically, the NS3 domain undergoes autocleavage in absence of GZV, thus abrogating any CAR signaling and CAR T cell function. In vitro, primary T cells expressing the anti-PD-1 CAR NS3 kill PD-1+ cells and secret IFNγ in presence of GZV in a dose-dependent manner indicating that the NS3 domain functions as an ON switch.

Conclusion: Combined, these data indicate that a drug-controlled anti-PD-1 CAR NS3 is highly functional and can be controlled exogenously through administration or withdrawal of GZV. These data warrant further investigation of drug-controlled anti-PD-1 CAR T cells in ART-treated SIVmac239-infected rhesus macaques to transiently deplete Tfh cells to determine their contribution to the latent reservoir.

535 Disrupting SIV Reservoir Seeding by Targeting Stemness Pathways in Rhesus Macaques
Inna Ruiz-Salinas1, Rizi R. Hamid2, Nils School3, Alice Lin4, Jordan Galdy5, Guido Silvestri5, Ann Chahroudi5, Maud Mavigne2
1Emory University, Atlanta, GA, USA, 2Emory National Primate Research Center, Atlanta, GA, USA

Background: Latently HIV-infected CD4+ T cells persist indefinitely through proliferation. We previously showed that inhibition of proliferation and induction of differentiation of the long-lived, self-renewing central (CM) and stem cell memory (SCM) CD4+ T cells can be achieved in ART-treated SIV-infected rhesus macaques (RMs) through modulation of the Wnt pathway. Here, we evaluated a combined approach targeting Wnt and Notch pathways during acute SIV infection of RMs to disrupt viral reservoir establishment.

Methods: Five RMs were infected i.v. with SIVmac239 before receiving 8 weeks of treatment with the Wnt inhibitor PRI-724 administered subcutaneously daily at 18-20 mg/kg in combination with the Notch inhibitor LY3039478 administered orally at 2.5 mg/kg three times per week. ART was initiated 8 weeks post-infection (wpi) and PBMC were collected longitudinally on ART to FACS sort subpopulations of naive, SCM, CM, transitional memory (TM) and effector memory (EM) CD4+ T cells. Levels of cell-associated total SIV gag DNA and unintegrated 2-LTR circles were measured by multiplex QPCR in sorted cells. A group of 7 RMs infected with SIVmac239 and treated with ART alone served as controls.

Results: The combined treatment PRI-724+LY3039478 demonstrated an acceptable safety profile and did not alter plasma viral load dynamics in SIV-infected RMs. After ART initiation, the decay in the SIV 2-LTR circles, that are diluted with proliferation, was greater in the CM (p=0.005 at 12wpi) and TM (p=0.018 at 12wpi, p=0.003 at 20wpi) CD4+ T cells from the PRI-724+LY3039478-treated RMs as compared to controls. The ratio of SIV gag/2-LTR was higher in all subsets of memory CD4+ T cells from the treated RMs versus controls at 8 (p<0.035) and 12wpi (p<0.073). Interestingly, a reduced contribution of CM to the total SIV reservoir in CD4+ T cells was observed in the treated RMs as compared to controls at both 8 and 12wpi (p=0.010 for both). This reduction was attributed to lower levels of SIV gag DNA in CM CD4+ T cells at 12wpi (p=0.048) and decreased frequencies of CM cells within the CD4+ T cell pool at 8 and 12wpi (p=0.004 and 0.018) in PRI-724+LY3039478-treated RMs versus controls.

Conclusion: This proof-of-concept study suggests that the combined pharmacological modulation of Wnt and Notch pathways during acute SIV infection of RMs impacts viral reservoir seeding by transiently reducing the relative contribution of the CM cells to the peripheral CD4+ T cell compartment and to the pool of the infected CD4+ T cells.
Impact of Gender-Affirming Hormone Therapy on HIV Reservoirs and Inflammation in Transgender Women

Elizabeth Hastie, Antoine Chaillon, Alan Wells, Christophe Vanpouille, Christy Anderson, Magali Porrachia, Kya Forshy, Vanessa Gomez-Moreno, Megha S. Srivatsa, Jill Blumenthal, Marin Hanashiro, Eileen P. Scully, Jordan E. Lake, Jonathan Karr, Sara Gianella

1University of California San Diego, La Jolla, CA, USA, 2National Institute of Child Health and Human Development, Bethesda, MD, USA, 3The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4University of Texas at Houston, Houston, TX, USA, 5Case Western Reserve University, Cleveland, OH, USA

Background: Transgender women (TW) are at increased risk for HIV but are underrepresented in research. We investigated how the initiation of gender affirming hormone therapy (GAHT) impacts HIV reservoir (size and activity) and inflammation.

Methods: TW with HIV starting estradiol-based GAHT were recruited at two clinics in San Diego, CA and Houston, TX. Participants were on antiretroviral therapy (ART) and had suppressed HIV-1 RNA during the study. Blood and plasma were collected prior to starting GAHT and longitudinally 2, 4, 6, 9, 12, and 18 months post GAHT initiation. Historical samples from cisgender men with HIV were matched by age, CD4+ T cell count, duration of HIV, and time on ART. At each timepoint, we measured estradiol and testosterone by ELISA, cell-associated HIV RNA (unspliced and multiple spliced) and HIV DNA (total and 2-LTR) by ddPCR, and 41 cytokine/chemokines by Lumines. We used a linear mixed effects model with HIV reservoir values as outcomes, and time, group, and their interaction as predictors along with a random intercept. Partial least squares-discriminant analysis (PLS-DA) was used to predict gender groups using cytokine/chemokines. Error rates were estimated using k-fold cross-validation.

Results: A total of 22 TW were enrolled. Nine were lost to follow-up including one suicide, one homicide, and two imprisonments. A total of 77 samples from TW and 79 samples from cisgender controls were analyzed. Compared to cisgender controls, TG women had higher overall estradiol (145.83 vs 45.62 pg/mL, p<0.001), and lower testosterone concentrations (226 vs. 436 ng/dL, p<0.001) with HIV were matched by age, CD4+ T cell count, duration of HIV, and time on ART. At each timepoint, we measured estradiol and testosterone by ELISA, cell-associated HIV RNA (unspliced and multiple spliced) and HIV DNA (total and 2-LTR) by ddPCR, and 41 cytokine/chemokines by Lumines. We used a linear mixed effects model with HIV reservoir values as outcomes, and time, group, and their interaction as predictors along with a random intercept. Partial least squares-discriminant analysis (PLS-DA) was used to predict gender groups using cytokine/chemokines. Error rates were estimated using k-fold cross-validation.

Conclusion: TW had increased HIV DNA with faster decline in 2-LTR circle after initiating GAHT compared to cisgender men, supporting estradiol’s inhibition of residual HIV replication. TW had unique immune signatures, which might impact HIV disease outcomes and comorbidities. Our study underscores the challenges associated with recruiting and studying this unique population, emphasizing the need for trauma-informed care and tailored research efforts.

Figure: (A) HIV 2-LTR and total HIV DNA over time; (B) Cytokine partial least squares-discriminant analysis projection with confidence ellipses.

Potent Indoline CD4 Mimetics Enable Anti-Coreceptor Binding Site Antibodies to Mediate ADCC

Jonathan Richard, Li Zhu, Lorie Marchitelli, Catherine Bourassa, William D. Tobert, Sri Lakshimi T. Boodapatii, Derek Yang, Hong Kim, Guillaume Beaudoin-Bussieres, Mehdi Benlarbi, Joseph Sodroski, Amos B. Smith III, Marzena Pazgier, Priti Kumar, André Finzi

1Centre de Recherche du CHUM, Montreal, Canada, 2Yale University, New Haven, CT, USA, 3Uniformed Services University of the Health Sciences, Bethesda, MD, USA, 4University of Pennsylvania, Philadelphia, PA, USA, 5Centre de Recherche du CHUM, Université de Montréal, Montreal, Canada, 6Harvard Medical School, Boston, MA, USA

Background: Antiretroviral therapy efficiently suppresses HIV-1 replication but does not eradicate the virus. New approaches aimed at eliminating infected cells represent an appealing avenue to achieve this goal. Non-neutralizing antibodies (nnAbs) naturally present in the plasma of people living with HIV-1 (PLWH) have the potential to eliminate HIV-1-infected cells via antibody-dependent cellular cytotoxicity (ADCC). However, these nnAbs largely recognize epitopes only accessible upon interaction of the envelope glycoproteins (Env) with CD4. HIV-1 limits surface Env-CD4 interaction by downregulating CD4, thus protecting infected cells from CD4–induced (CD4) nnAb–mediated ADCC responses. CD4-mimetic compounds (CD4mics) can “open-up” Env and sensitize HIV-1–infected cells to ADCC mediated by HIV-1+ plasma. We have shown that two families of CD4 nnAbs contribute to eliminate infected cells in the presence of CD4mic: the anti-cluster A and the anti-coreceptor binding site (CoRBS) Abs. Of note, while indoline CD4mics significantly enhance the recognition of infected cells by anti-CoRBS Abs, these nnAbs mediate ADCC poorly. The combination of anti-CoRBS Abs together with CD4mic and anti-Cluster A is required to mediate ADCC. This combination reduces the size of the HIV-1 reservoir and delays viral rebound after ART interruption in humanized mice. The development of new indoline CD4mic with improved neutralization potency and breath enabled us to revisit the capacity of anti-CoRBS Abs to mediate ADCC.

Methods: The capacity of anti-CoRBS Abs to mediate ADCC against HIV-1–infected cells in the presence of indoline and indoline CD4mics was assessed in vitro, while their capacity to delay viral rebound in vivo was evaluated in infected humanized mice.

Results: Contrary to indoline CD4mics, CDF–III–288, sensitizes HIV-1–infected cells to ADCC mediated by anti-CoRBS Abs. This was observed with multiple HIV-1 primary strains and using various anti-CoRBS Abs. Accordingly, treatment of humanized mice with CDF–III–288 and an anti-CoRBS Ab alone was sufficient to delay viral rebound after ART interruption, and notably, for much longer when administered prior to ART initiation. These results suggest that the indoline CD4mic open the Env trimer in such a way that favors Fc gamma receptor engagement on effector cells.

Conclusion: More potent indoline CD4mic have the ability to sensitize HIV-1–infected cells to CoRBS Abs-mediated ADCC responses, thereby improving their therapeutic potential.

Lipid Composition and CXCR4 Decoration Facilitate HIV-1 CRISPR Delivery and Viral Excision

Sudipta Panja, Lubaba A. Zaman, Milankumar Patel, Howard E. Gendelman University of Nebraska Medical Center, Omaha, NE, USA

Background: A principal limitation in achieving HIV cure rests, first, in locating and then eliminating integrated proviral DNA from viral target cells (CD4+ T and myeloid cells). In recent reports, the development of viral vector to deliver clustered regularly interspaced short palindromic repeats (CRISPR) guide RNAs (gRNAs) provided a proof of concept that HIV-1 elimination could be achieved. Our goal is to extend and improve those results by creating: (1) multiple viral exons (gp41, tat, and rev) gRNAs; (2) viral tissue reservoir targeted lipid nanoparticles (LNPs); and (3) decorating LNPs with CXCR chemokine receptor-4 (CXCR4) for targeting cellular reservoir. The biodistribution of LNPs was evaluated in humanized mice. We posited that effective gRNA delivery and, subsequently, viral DNA elimination can be achieved with our approach.

Methods: A library of gRNAs was designed to disrupt five HIV-1 exons (tat1-2/rev1-2/gp41). These were derived from a consensus sequence of the transcriptional regulator tat from 4004 HIV-1 strains. CXCR4-targeting cyclic-peptide (CycPep) was conjugated with PEG-lipid to make DSPE-PEG-CycPep and LNP was formulated by microfluidic mixing. The specificity of the CXCR4 receptor was shown by blocking the CXCR4 receptor to demonstrate lymphoid targeting. CRISPR gRNA delivery and excision were tested in lymphocytic (JLat
Retinoids Enhance NK Natural and Antibody-Dependent Cell Cytotoxicity of HIV-infected CD4 T-Cells

Elyse K. McMahan, Natalie Howard, Rebecca M. Lynch, Alberto Bosque
George Washington University, Washington, DC, USA

Background: Novel approaches to sensitize latently infected cells to apoptosis may provide additional methods to eliminate latent reservoirs. Prior research identified several retinoids as potential drugs that increase the sensitivity of HIV-infected cells to cell death. Retinoids are derivatives of Vitamin A that target retinoid receptors causing antiproliferative and proapoptotic activity. Several are FDA-approved or in clinical trials for different etiologies. The aim of this study was to evaluate the ability of retinol, 3 of its natural metabolites and 9 synthetic derivatives to sensitize HIV infected CD4 T cells to NK cell killing.

Methods: naive CD4 T cells isolated from PBMCs from male and female donors were activated, expanded and then infected with the replication competent HIV molecular clone NL43. CD4 T cells were cocultured overnight with autologous NK cells in the presence of 1μM of a retinoid and antiretroviral therapy, with or without 100ng/mL of IL15. We used three retinoids: alitretinoin, tazarotene and AM80 to assess their ability to enhance Antibody-Dependent Cell Cytotoxicity (ADCC) by using the same procedure with the broadly neutralizing antibody N6 (μg/mL). To assess the mechanisms by which these retinoids enhance NK killing of HIV-infected cells, we measured changes of MHC Class I receptor expression on CD4 T cells and cytotoxicity markers on NK cells.

Results: None of the retinoids were toxic alone or with IL15. Without IL15, none of the retinoids influenced NK natural cytotoxicity. With IL15, alitretinoin (n=8, p<0.001), tazarotene, tazarotene acid and AM80 (n=8, p<0.05) significantly enhanced natural cytotoxicity of HIV-infected cells compared to DMSO control. Mechanistically, these four retinoids increased NK degranulation upon target recognition in the presence of IL15 (n=8, p<0.05). In initial studies, all three retinoids enhance HLA-F expression on CD4 T cells, previously known to enhance recognition by NK cells. Furthermore, these three retinoids significantly enhanced the ability of N6 to induce ADCC compared to DMSO control regardless of IL15 by increasing CD16 expression on NK cells (n=8, p<0.001).

Conclusion: We identified three retinoids capable of enhancing HIV-infected cells to NK natural cytotoxicity and ADCC. We are conducting experiments to confirm the mechanisms associated with this phenotype. Our studies may provide further evidence of small molecules that could be used clinically to reduce persistent reservoirs.

Comparison of Anti-HIV ADC and Immunotoxin Shows Clinically Relevant Differences in Cytotoxic Effect

Seth H. Pincus, Tami Peters, Megan Stockhouse, Kelli Ober, Frances Cole, Hans-Peter Kiem, Robert Harrington, Xinyi Wang, Anne-Sophie Rahmann, Valérie Copié
1Montana State University, Bozeman, Montana, USA, 2Walter Reed Army Institute of Research, Silver Spring, MD, USA, 3Sofia Casares, 4George Washington University, Washington, DC, USA, 5Naval Medical Research Center, Silver Spring, MD, USA, 6University of Louisiana at Lafayette, Lafayette, LA, USA

Background: The persistent reservoir of cells carrying a functional provirus is the barrier to HIV eradication. One approach to reservoir depletion is sequential activation of latent virus, followed by purging of HIV-expressing cells. initially it was hoped that once cells were activated, viral cytopathic effect or host immune responses would clear the reservoir, but recent studies using mAbs have shown greater effects. We have armed antibodies with cytotoxic agents to enhance killing of HIV-infected cells, independent of ADCC or complement activity. We screened >200 mAbs to identify those most effective at delivering the toxic payload, and found that the anti-gp41 mAb 7B2 conjugated to ricin A chain, is safe and effective in SHIV-infected macaques. However, it was immunogenic, limiting its utility. We therefore sought to develop less immunogenic immunotoxins (ITs) and antibody drug conjugates (ADCs).

Methods: We screened drugs for cytotoxicity on resting lymphocytes. ADCs were prepared by chemical conjugation to 7B2. Cytotoxicity of ADCs was compared to ITs on Env-expressing cells. Antiviral effects were measured in tissue culture. The effect of ADCs and ITs on metabolism and transcription in HIV-infected cells was studied by NMR-based metabolomics and RNAseq.

Results: PNU-159682, an anthracycline, killed resting cells. We compared 7B2-PNU (ADC) to 7B2-ricin A (IT) in vitro. Cytotoxicity of the IT was 10X greater than the ADC on Env+ target cells, whereas neither IT nor ADC killed Env- cells. The ADC required >24 hr to initiate cytotoxicity; IT killing began within 6 hr. The ADC elicited bystander killing of Env- cells when mixed with Env+ cells, the IT did not. At 6 hr, metabolic analyses of IT-treated cells, but not ADC-treated or untreated cells, demonstrated elevated amino acids and choline and depressed taurine, creatine, and fumarate. At 24 hr the metabolic profiles of all three groups diverged. Similarly, the transcriptome of IT-treated cells diverged at 6 hr, while at 24 hrs, the three transcriptional profiles were distinct. Pathway analysis indicated significant up-regulation of transcriptional control and death pathways in both IT and ADC treated cells.

Conclusion: Cytotoxic immunotoxins (ITs) targeted by anti-gp160 mAbs are potent killers of cells expressing HIV Env, work independently of Fc-effector functions, and are potentially useful in HIV eradication. Understanding how different ITs kill cells will guide their clinical application.
542 Type I IFN Signaling and Regulation in Vesatolimod-Treated Virally Suppressed Adults With HIV-1
Susie S. Huang, Liao Zhang, Donovan Verrill, Christiaan R. de Vries, Elena Vendrame, Devi SenGupta, Jeff F. Wallin, Yanhui Cai
Gilead Sciences, Inc, Foster City, CA, USA

Background: Vesatolimod (VES) is a well-tolerated and selective toll-like receptor-7 agonist under development as part of an HIV cure regimen. VES treatment increases interferon-stimulated gene (ISG) expression and immune cell activation in people with HIV (PWH); additionally, VES monotherapy promotes a modest delay in HIV rebound in HIV controllers, and VES pharmacodynamic response is higher with 8 mg compared with 10-12 mg doses in PWH treated during chronic infection. We used mRNA-Seq to further investigate the immune mechanism and regulation of type I interferon (IFN) signaling pathways in response to VES in a phase Ib, double-blind, placebo-controlled trial (NCT02858401; GS-US-382-1450).

Methods: The study enrolled virally suppressed PWH (age ≥ 18 years, plasma HIV-1 RNA <50 copies/ml) and randomized 6:2 to receive VES or placebo biweekly for 10 doses. Whole blood mRNA collected pre-dose and 24 hours after doses 1 and 10 from participants who received VES 6 mg, 8 mg, or 10-12 mg (n=24) was used for mRNA-Seq (Illumina Stranded mRNA Prep; NovaSeq 6000). A linear mixed-effects and placebo-adjusted model was used to test for differentially expressed genes (DEGs) associated with type I IFN regulation and patterns of regulator recognition (FDR <0.1).

Results: VES consistently upregulated DEGs associated with type I IFN signaling after doses 1 and 10 in all 3 doses evaluated (Figure). After dose 1, the highest number of DEGs, including those associated with activation and inhibition, was observed with VES 8 mg (38 genes), followed by VES 6 mg (21 genes) and VES 10/12 mg (15 genes); type I IFN activation was most pronounced with 8 mg versus the other doses. After dose 10, VES 6 mg induced the highest type I IFN activation and numbers of genes (49), followed by VES 8 mg (31) and VES 10/12 mg (27), respectively. Also, a lower induction in type I IFN activation was observed by post dose 10 versus post dose 1 in all but the 6 mg dose group, with the greatest reduction in the 8 mg group. VES-mediated type I IFN activation was greatest after dose 1 at 8 mg, followed by dose 10 at 6 mg.

Conclusion: Whole blood transcriptome analysis identified pronounced differences in gene expression based on VES dose and administration order. The results suggest that a cumulative effect of multiple VES doses could modulate VES pharmacodynamic response, which should be taken into account in future combination studies with VES.

543 Evaluation of a Bispecific Antibody Targeting NK Cells for the Elimination of HIV Reservoirs
Nerea Sanchez Gaona, David Pérez, Adrià Curran, Joaquín Burgos, Jordi Navarro, Paula Suárez, Vicenç Falco, Elena Martín Gayo, Meritxell Genesca, Jorge Carrillo, Maria Buzón
Vall d’Hebron Research Institute, Barcelona, Spain; 1Hospital Universitario de la Vall d’Hebron, Barcelona, Spain; 2Universidad Autónoma de Madrid, Madrid, Spain; 3Instituto for AIDS Research, Badalona, Spain

Background: “Shock and kill” strategies for HIV cure involve reactivating latent virus (i.e. using latency reversal agents), followed by immune-mediated clearance. HIV impairs NK function, hampering their ability to eliminate infected cells, highlighting the need for immunotherapies to enhance NK cytolytic activity against HIV.

Methods: We designed a tetravalent bispecific antibody (Bi-Ab32/16), which targets the gp120 HIV protein (Ab A32) and CD16a. We assessed its functionality through ADCC and cytotoxicity assays (n=7). Killing of viral reactivated cells (n=4) and changes in the intact proviral DNA (n=5) were evaluated in CD4+ T cells from ART-treated PWH. Preliminary evaluation was conducted in humanized mice expressing ILS (NSG-Hu-IL15). HIV-infected mice received ART from 6 to 9 weeks post-infection (wpi) and were randomized (n=6 per group) to receive immunotherapy at 10 mg/kg (Bi-Ab32/16 or A32) from 8 to 10 wpi. Mice were euthanized at 11 and 13 wpi. Immune subsets and NK activation were assessed by flow cytometry. HIV RNA was quantified by RT-qPCR.

Results: Bi-Ab32/16 enhanced NK activation, degranulation, and cytotoxicity against HIV-infected cells in vitro (p<0.05). Further, the EC50 (0.01±0.003 µg/ml) was 55-fold lower than the parental A32 Ab (0.55±2.5E-5 µg/ml). Following viral reactivation, Bi-Ab32/16 improved killing of HIV-infected cells (median reduction 55%, p<0.01), and mediated the clearance of cells harboring intact proviruses in 1/5 PLWH (median reduction 50.7%). HIV viral loads were detected at 3 wpi (1010±10 copies/ml) in 24/34 animals. HIV-infected mice showed decreased CD4 counts and CD4/CD8 ratios (p<0.05), and increased frequencies of CD16+ NK (p<0.001), which were all normalized after ART initiation (p<0.001). Higher IFNγ-expressing NK memory-like subsets were observed at 13 wpi with Bi-Ab32/16 compared to A32 and control groups (p<0.05). However, Bi-Ab32/16 reduced CD16+ NK counts (p<0.05) (Fig.1A), which was inversely associated with infection after ART interruption (p<0.05). Furthermore, Bi-Ab32/16 animals had a shorter time to viral rebound versus A32 and control groups (p<0.05) (Fig.1B).

Conclusion: Bi-Ab32/16 activates NK, enhances ADCC, and diminishes latent HIV infection after viral reactivation in samples from PLWH. However, persistent in vivo NK stimulation with Bi-Ab32/16 reduces CD16+ NK, negatively impacting HIV control. Cellular in vivo therapies involving NK preloaded with Bi-Ab32/16 might offer a promising strategy for HIV elimination.

Fig.1. Preliminary evaluation of Bi-Ab32/16. A) Evaluation of CD8+CD16+NK frequencies by t-test and group. Mann-Whitney U test. *p<0.05. **p<0.01. B) Kaplan-Meier curve depicting the proportion of animals experiencing viral rebound following ART discontinuation, stratified by group. Log-rank (Mantel-Cox) test. *p<0.05.
Associations Between Nadir CD4 and Subsequent Immune Measures With Long-Term Cognitive Function

Frank Pelleda,1 Janeway Grancheb,1 Douglas W. Kitchc,1 Katherine Tassiopoulousb, Susan L. Koletar1

Northwestern University, Chicago, IL, USA, 1Harvard TH Chan School of Public Health, Boston, MA, USA, 3The Ohio State University, Columbus, OH, USA

Background: Relationships between immune measures and neurocognition in persons with HIV (PWH) on ART are unclear.

Methods: We analyzed data, including nadir and current CD4 cells/µl (CD4), CD4%, and CD4/CD8 ratios, from virally suppressed PWH (HIV RNA <200 copies/ml >75% of time since ART start) with >2 NPZ3 scores in ALLRT (ACTG 5001) and HAILO (ACTG 5322). We used longitudinal linear mixed models with fixed and random effects to assess associations between NPZ3 (measured ≥96 weeks post ART start, baseline) and immune measures. We adjusted models for age, sex, and race/ethnicity, with and without adjustment for HIV RNA (>200 vs ≤200 copies/ml), type and years of ART use, and HCV infection.

Results: Among 885 PWH seen 1/1/2000-12/30/2021, median (Q1, Q3) age at ART start was 44 (39, 48); 81% were male, 50% were White, 29% Black, 21% Hispanic or other. CD4 nadir was 0-50 (22%), 51-200 (29%), 201-350 (33%), 351-500 (11%), 501+(5%). Baseline median CD4 was 486, CD4:CD8 0.66 and NPZ3 score -0.10 (Q1) -0.70 (Q3), 0.53. Higher nadir CD4 was associated with higher baseline NPZ3 but lower NPZ3 increase: PWH with nadir CD4 ≤50 had mean NPZ3 increases of 0.067 per year (95% CI= [-0.056, 0.077]); PWH with nadir CD4>501 had smaller increases (0.030 fewer points) per year (95% CI= [-0.054, -0.007]). Adding any time-updated CD4 measure attenuated nadir CD4 associations with NPZ3, often rendering them non-significant. A standard deviation (1SD) (309 cells/µl) increase in maximum CD4 was associated with an increase in NPZ3 score of 0.034 (95% CL= -0.011, 0.080), and a reduction in NPZ3 score slope of -0.007 (-0.012, -0.002). When nadir and current CD4% were analyzed, nadir CD4% had the strongest associations with current NPZ3 score and change over time, but greater increases over time. Current CD4% and CD4:CD8, but not absolute CD4, remained most strongly associated with current NPZ3 score and change over time.

Conclusion: Lower nadir CD4 was associated with lower NPZ3 score at baseline but greater increases over time. Current CD4% and CD4:CD8, but not absolute CD4, had the strongest associations with current NPZ3 score and change over time.

Predictors of CD4/CD8 T-Cell Inversion After 96 Weeks of ART Initiated During Acute HIV

Robert Paul,1 Kyu Cho,1 Carlo P. Sardalan,2 Ferron F. Ocampo3, Phillip Chan4, Lydie Trautmann5, Julie Ake5, Somchai Sripluenchan,6 Nathornn Psrutrum,7 Jacob Bolzenius8, Sandhya Vasan9, Napapon Saisailait10, Kilian Pohl11, Serena S. Spudich12, for the RV254/SEARCH010 Study Team

1University of Missouri St Louis, St Louis, MO, USA, 2SEARCH Foundation Research, Bangkok, Thailand, 3Yale University, New Haven, CT, USA, 4US Military HIV Research Program, Silver Spring, MD, USA, 5Walter Reed Army Institute of Research, Silver Spring, MD, USA, 6SEARCH, Bangkok, Thailand, 7University of Hawaii at Manoa, Honolulu, HI, USA, 8Stanford University, Stanford, CA, USA

Background: >50% of people with HIV (PWH) do not achieve CD4/CD8 T-cell ratio normalization (ratio>1.0) on antiretroviral therapy (ART). Recent findings from RV254/SEARCH010, a longitudinal investigation of acute HIV infection (AHI) and long-term response to ART, identified a combination of immune and behavioral factors (e.g., mental health) that predicted CD4/CD8 T-cell ratio inversion at week 144 of ART. This study examined a larger array of potential predictors of CD4/CD8 T-cell inversion, including multimodal indices of brain structure/function quantified using 3T MRI before ART initiation.

Methods: Archival data from RV254/SEARCH010 were examined for individuals who completed neuroimaging (volumetrics, diffusion tensor imaging (DTI), and resting state connectivity) at enrollment followed by 144 weeks of sustained ART. Gradient boosted multivariate (GBM) regression with repeated cross validation was utilized to identify predictors for persistent CD4/CD8 T-cell ratio inversion at week 144 from baseline neuroimaging, demographic, immune, viral, cognitive, and mental/behavioral health indices.

Results: 74 Thai males with an average duration of HIV infection of 19.5 (7.2) days were included in the analysis. Study participants were of 27.4 (6.0) years of age and had a median viral load (log10) ≥6.01 (IQR 5.43-6.79) copies/ml, CD4+ T-cell count of 330, CD8+ T-cell count of 526, and CD4/CD8 T-cell ratio of 0.64. After 144 weeks of ART, all study participants were virally suppressed. 34 individuals had a CD4/CD8 T-cell ratio <1.0. The GBM analysis identified 10 baseline features that predicted CD4/CD8 T-cell ratio inversion at 144 weeks (average model performance of 0.72). The algorithm included larger volumes in 6 brain regions (medial superior frontal gyrus, superior temporal gyrus, rectus gyrus, nucleus accumbens, cerebellar lobes IV-V, pallidum), lower connectivity between the salience and visuospatial network, lower high-density lipoprotein (HDL) levels, lower radial diffusivity in the external capsule, and higher IL-1α.

Conclusion: Perturbations in brain structure and function, possibly reflecting early inflammatory mechanisms, dyslipidemia, and inflammation prior to ART commenced in acute infection stratify individual risk for persistent CD4/CD8 T-cell ratio inversion following sustained use of ART. Further investigation of potential causal pathways is needed to establish mechanisms and therapeutic opportunities.

Assessing Blood-Based Biomarkers as Predictors of Cognitive Decline in PWH: ACTG A5322 (HAILO)

Shiabi S. Mukerji1, Petra Bachanová1, Linzy V. Rosen2, Hemi Park2, Rommi Kashlan1, Pia Kinisakkı, Felicia C. Chow1, Kunling Wu3, Raha M. Dastgheyb4, Leah H. Rubin1, Katherine Tassiopoulousb, Robert A. Parker5, Emily P. Hyle1

1Harvard Medical School, Boston, MA, USA, 2Massachusetts General Hospital, Boston, MA, USA, 3University of California San Francisco, San Francisco, CA, USA, 4Harvard TH Chan School of Public Health, Boston, MA, USA, 5Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: Blood-based markers (BBMs) show promise in evaluations of Alzheimer’s disease and other cognitive disorders. We examined plasma neurofilament light chain (NFL) and glial fibrillary acid protein (GFAP) and their
relationship to cognitive function in people with HIV (PWH) enrolled in the ACTG aging cohort study, HALO.

**Methods:** Clinical data and plasma were obtained from 550 PWH aged ≥45y with ≥2 neuropsychological tests (NP) and HIV RNA<200 copies/ml. Four NP scores (Trail Making Test Part A and B, Digit Symbol, and Hopkins Verbal Learning Test-Revised) were standardized as z-scores. The average of these z-scores (NPZ4) at plasma collection or subsequent visits were provided by HALO and used as primary outcomes; longitudinal scores were summarized by calculating the slope for each participant. We used a single molecule array platform to quantify NFL and GFAP. Associations between BMI and baseline NPZ4 scores or slopes were assessed with linear regression. Longitudinal analyses were done for the total cohort and a subset with NPZ4 decline (slope <0). Models were adjusted for age, sex at birth, race, and years of education; regressions of slopes were also adjusted for baseline NPZ4 and weighted by number of visits.

**Results:** Mean age was 53y (range 45-77y), and observation time was 6.2y, 19.6% were female, and 28.4% non-Hispanic Black. Median [IQR] baseline NFL, GFAP, and NPZ4 scores were 10.7 pg/ml [7.98, 14.62], 77.5 pg/ml [58.80, 107.86], and 0.11 [-0.48, 0.75], respectively; median [IQR] NPZ4 slope was 0 [-0.06, 0.06]. In adjusted models, higher NFL or GFAP was associated with worse baseline NPZ4 (β=0.05 per 10pg/ml NFL; p=0.06, -0.14 per 100 pg/ml GFAP; p=0.04) and became significant after excluding outliers NFL≥25 (p=0.03) but not GFAP≥200 (p=0.2). While baseline NFL (p=0.11) and GFAP (p=0.05) showed a weak relationship with NPZ4 slope in the total cohort, NFL had a stronger association in the subset with cognitive decline (n=263; -0.02 annual decrease per 10pg/ml NFL, p<0.01; Figure 1). The variance explained was ≤10% in all models.

**Conclusion:** In this cohort of PWH, with the majority in mid-life and cognitively stable, NFL and GFAP were associated with a minor decrement in cross-sectional NPZ4, and NFL was weakly associated with longitudinal NPZ4. NFL more strongly predicted NPZ4 slope in a prespecified subset with cognitive decline. Overall, results highlight the limitations of NFL and GFAP as predictive biomarkers of cognitive decline in this age range.

**Figure:** Relationship between microglial light chain (NFL) and slope for longitudinal NPZ4 in PWH aged 45y. The scatter plot shows the regression line with 90% confidence band around the predicted value between [NFL slope] and [baseline NFL]. Each dot represents a participant, and color denotes age.

454 Inflammation and Cognition Associations in Young Adults With Perinatal HIV Exposure and/or Infection
Megan S. McHenry1, Yanling Huo2, Paige L. Williams1, Kanjal Patel3, Wei L4, Alka Khaitan1, Sharon Nichols2, for the Pediatric HIV/AIDS Cohort Study (PHACS) Network. 1Indiana University, Indianapolis, IN, USA; 2Harvard University, Cambridge, MA, USA; 3Harvard Chan School of Public Health, Boston, MA, USA; 4University of California San Diego, La Jolla, CA, USA

**Background:** Children with perinatal HIV exposure, particularly those with HIV infection, are at risk for worse cognitive outcomes compared to unexposed children. Inflammation is a potential risk factor for worse cognitive outcomes in populations with HIV, but no studies have evaluated this association within young adults with perinatal HIV (YAPHIV) or perinatal HIV exposure but uninfected (YAPHEU). We evaluated the association between inflammatory biomarkers and NIH Toolbox cognitive outcomes in YAPHEU and YAPHIV.

**Methods:** Participants with available plasma samples and NIH Toolbox cognitive scores at entry into the PHACS AMP Up Study were included in the analysis. Principal Component Analysis (PCA) was used to derive factor scores from biomarkers for monocyte activation (CD14n, CD163), acute phase inflammation (CRP, fibrinogen, TNF-α), proinflammatory/Th1/Th17 (IFN-γ, IL-1β, IL-2, IL-12p70, IL-17a), or anti-inflammatory/Th2 (IL-4, IL-6, IL-10) cytokines and homeostasis (GM-CSF, Fractalkine). We computed Pearson correlations of individual log-transformed biomarkers and four PCA factors with Composite Standard Scores for Total (TC), Fluid (FC); e.g., processing speed, executive functions), and Crystalized Cognition (CC; e.g., word knowledge), separately by HIV status.

**Results:** 638 participants (YAPHEU: n=521, mean age 22.7 (SD:4.2) years; YAPHIV: n=117, mean age 19.0 (SD:1.4) years) were included. Mean TC, FC, and CC were similar for YAPHEU and YAPHIV. Compared to YAPHEU, YAPHIV had higher levels of TNF-α, sCD14 and sCD163 and lower levels of IL-2, IL-4, IL-6, IL-10, IL-1β, IL-12p70, IL-17a, Fractalkine, and GM-CSF. YAPHEU had weak negative correlations of FC with CRP, sCD163, and Fibrinogen (r = -0.08 to -0.09) and of CC and TC with sCD163 (r = -0.09). YAPHEU had positive correlations of FC with IL-2, IL-12p70, and IL-17a (r = 0.18 to 0.24) and negative correlations of CC with Fractalkine and IL-10 (r = -0.17). Factor 3 (primarily sCD14, CRP, Fibrinogen) was weakly negatively correlated with both FC and TC in YAPHEU (r = -0.10) and Factor 1 (primarily GM-CSF, Fractalkine, IL-2, IL-1β, IL-12p70, IFN-γ, IL-17a) was positively correlated with FC in YAPHEU (r = 0.19) (Table).

**Conclusion:** Cognition weakly correlated with different summary PCA factor scores in YAPHEU (monocyte and acute inflammation) and YAPHIV (Pro-inflammatory/Th1/Th17 cytokines). Further research is needed to understand the role of inflammatory profiles, perinatal HIV exposure or infection and other factors in cognition.
549 Pro-Inflammatory Glycomic Dysregulations Define HIV-Associated Neurocognitive Impairments

Leila B. Giron1, Janeway Ganche2, Frank Palletta3, Katherine Tassiopoulos4, Mohamed Abdel-Mohsen1

1Wistar Institute, Philadelphia, PA, USA, 2Harvard TH Chan School of Public Health, Cambridge, MA, USA, 3Northwestern University, Chicago, IL, USA, 4Harvard TH Chan School of Public Health, Boston, MA, USA

Background: In the general population, host glycomic alterations drive inflammation and precede onset of inflammation-associated diseases. However, it remains unclear whether glycomic alterations are associated with development of inflammation-associated diseases, including neurocognitive impairment, in people with HIV (PWH) on suppressive antiretroviral therapy (ART).

Methods: In this analysis conducted within the ACTG A5322 (HAILO), 20 PWH on ART (10 men and 10 women) who were cognitively impaired over 8 years of follow-up were matched by sex, age, and ethnicity to 20 controls without impairment. Cognitive function was assessed using the Trail Making A and B tests, the Wechsler Adult Intelligence Scale-Revised Digit Symbol test, and the Hopkins Verbal Learning Test—Revised. NPZ4 scores, calculated as the mean of these test scores. Cognitive impairment was defined as ≥2 z-scores ≤1 SD from the normative mean or one z-score ≤2 SD from the mean. Longitudinal samples, collected over 8 years (5-9 per participant), were analyzed for 133 IgG and plasma glycans (using capillary electrophoresis) and 10 inflammation markers (using multiplex arrays). Longitudinal associations between neurological impairment or NPZ4 scores and glycans and inflammation markers were assessed using mixed-effects models.

Results: Cognitive impairment was associated with higher levels of total bisected GlcNAc glycans, as well as several glycomic traits containing bisected GlcNAc, such as (G0FB and G1FB) on IgGs, pro-inflammatory glycans that increase with age (Fig. 1A). Higher NPZ4 scores were associated with lower levels of these glycans (Fig. 1B). Consistent with their pro-inflammatory roles, these glycans, specifically G0FB, correlated with higher levels of the inflammatory marker TNFα (P<0.01; rho=-0.44). Conversely, cognitive impairment was associated with lower levels, and NPZ4 scores with higher levels, of glycans that contain the anti-inflammatory sialic acid and fucose (such as FA2G2S1 and FA2G2S2). Finally, among inflammation markers, IL-10 and sCD14 exhibited positive correlations with neurocognitive impairment and negative correlations with NPZ4 scores.

Conclusion: Aging- and inflammation-associated host glycomic dysregulations are linked to the presence of neurological impairments in PWH on ART. Future studies are warranted to validate these exploratory findings and to examine potential prognostic and functional significance of host glycans and inflammation markers in the pathogenesis of neurological impairments in PWH. The figure, table, or graphic for this abstract has been removed.

550 WITHDRAWN

551 Immune Checkpoint Signatures Associated With Impaired Cognition in People Living With HIV

Ana Joy Lozano1, Christian Francisco2, Marissa Alejandro3, Glen Chew4, Chathura Siriwatharana5, Njoki Gondwana6, Robert Paul7, Cecilia Shikuma8, Lishomwa Ndhlovu9

1University of the Philippines Manila, Manila, Philippines, 2University of Hawaii, Honolulu, HI, USA, 3University of Missouri St Louis, St Louis, MO, USA, 4Weill Cornell Medicine, New York, NY, USA

Background: HIV-associated brain injury (HABI) persists among people with HIV (HIV+) despite suppressive antiretroviral therapy (ART). Immune checkpoint (IC) interactions have been associated with HIV comorbidities in PWH on ART, and IC blockade has been shown to restore immune function in the field of Oncology. Given the absence of effective interventions for HABI, we assessed for relationships with measures of IC pathways and neurocognitive deficits in PWH on suppressive ART.

Methods: A cross-sectional study of PWH (n=50) living on stable ART (≥12 months) with undetectable plasma HIV RNA (<50 copies/ml) and HIV-uninfected (n=50) demographically-matched controls were recruited in Metro Manila, Philippines to participant in the study and provide blood and undergo cognitive assessments. We assessed expression of ICs (PD-1, TIGIT, TIM-3, LAG-3) and their cognate ligands on immune cells using multiparametric flow cytometry. Neuropsychological (NP) performance tests were conducted and assessed for association with ICs and ligand expression. Non-parametric correlations between the two groups were performed and Spearman’s correlations.

Results: We observed an expansion of single and multiple ICs on both CD4 and CD8 T cells in PWH compared to HIV-uninfected controls. No differences were observed in cognate ligand expression. Only verbal memory and fine motor performance tests were impaired in PWH compared to controls (p<0.05). Higher CD4+ T cell frequencies correlated with better verbal memory (r=0.41, p=0.003) and worse fine motor (r=-0.39, p=0.005) performance, while higher TIGIT+ CD4+ T cells correlated with worse verbal memory (r=-0.35, p=0.01) in PWH but not in the control group. Given that neuronal cells have been known to express PD-L1 and PVR, a known TIGIT ligand, we further confirm the expression of PD-L1 and PVR on neuroblastoma cells and assessed the interaction of IC-expressing CD4+ T cells with neuroblastoma cells. We observed that PD-THigh CD4+ T cells in vitro increased PD-L1 expression on neuroblastoma cells and this was associated with an increase in TNF-α production. PD-L1 blockade reduced neuronal expression of PD-L1 but was insufficient to reverse the inflammatory response.

Conclusion: Our findings suggest that interactions of immune checkpoints on T cells with neuronal cells may drive a neuroinflammatory process leading to cognitive deficits in PWH despite viral suppression and additional intervention beyond IC blockade may be necessary.
Changes in Cognition, Mood, and Sleep Following EFV-DTG Switch in Virally-Suppressed PWH

Ronald J. Ellis1, Florin Vaida2, Keren Hu1, Michael Dubie2, Brook Henry1, Felicia C. Chou1, Lee Daniel1, Fred R. Sattler1
1University of California San Diego, La Jolla, CA, USA, 2AIDS Healthcare Foundation, Los Angeles, CA, USA

Background: In people with HIV (PWH) who are virologically suppressed (VS) on antiretroviral therapy (ART), abdominal obesity (AO) is associated with neurocognitive impairment (NCI). The likely mechanisms involve visceral adiposity, inflammation, and reductions in insulin-like growth factor type 1 (IGF-1), a neurotrophin. Tesamorelin (Tesa) is a synthetic growth hormone-releasing hormone that reduces AO and increases IGF-1, suggesting it may mitigate NCI in VS PWH.

Methods: We conducted a randomized clinical trial of immediate versus delayed treatment with Tesa with NCI in PWH. Entry criteria were PWH with VS, NCI (global deficit score [GDS] >0.5 on a comprehensive NC battery), and AO as indexed by elevated waist circumference (WC). Exclusions were other confounding conditions other than HIV accounting for NCI, active substance use disorder, and active malignancy. Participants were randomized 3:2 to receive either immediate or delayed Tesa 2 mg SC for 6 months. The primary outcome was the change in NC performance at 6 months by the published summary regression-based change score (sRCS) method, which corrects for the effects of repeated testing.

Results: We enrolled 73 participants who met the entry criteria. Per the study design, 43 were randomized to immediate Tesa and 30 to delayed treatment. The groups were well matched on baseline characteristics as follows: mean (95% CI) baseline GDS 0.939 (0.717, 1.161) in the immediate and 0.861 (0.653, 1.069) in the delayed Tesa arms, p=0.619. Mean WC was 111.0 (108.0, 114.0) cm in the immediate and 110.0 (106.1, 113.8) cm in the deferred Tesa arms, p=0.655. The immediate but not deferred Tesa arm showed improved NC performance, mean (95% CI) 0.155 (0.001, 0.309), p=0.048 in immediate, 0.103 (-0.095, 0.301), p=0.295 in deferred Tesa, but the difference for immediate Tesa compared to no treatment (delayed initiation) was not significant (p = 0.673). The immediate arm showed a higher reduction in WC at 6 months compared to the delayed arm (median difference: -2.7 [-4.7, -0.7] cm, p=0.015).

Conclusion: These findings indicate that Tesa did not significantly improve NCI in VS PWH with AO compared to delayed initiation, but the study was underpowered to confidently show a relationship. Other limitations were that the study was unblinded and did not include a concomitant placebo arm. These findings do not support the hypothesis that AO contributes significantly to NCI in PWH.

Changes in Cognition, Mood, and Sleep Following EFV-DTG Switch in South Africa: The CONNECT study

Sam Nightingale1, Anna I. Dreyer1, Kevin Thomas1, Gert U. van Zyl2, Eric Desloët2, Pieter Naude3, Catherine Orrill4, Phumla Simzadi4, Alan Winston4, Saye Kho4, John Joska1
1University of Cape Town, Cape Town, South Africa, 2Stellenbosch University, Cape Town, South Africa, 3Imperial College Healthcare NHS Trust, London, United Kingdom, 4University of Liverpool, Liverpool, United Kingdom

Background: Both efavirenz (EFV) and dolutegravir (DTG) have been associated with neuropsychiatric side-effects. Rates of cognitive impairment and CSF escape have not been comprehensively studied in African populations, and the effect of EFV-DTG switch on cerebral function is not known.

Methods: 178 virologically suppressed PWH on EFV-based ART were studied at baseline, and 145 followed up at 1-year following switch to DTG. 95 people without HIV (PWoH) were recruited from the same area, matched for age and sex, and 40 followed up at 1-year. Participants underwent comprehensive cognitive testing over 7 domains, measures of mood (Center for Epidemiologic Studies Depression Scale (CESD)), anxiety (State-Trait Anxiety Inventory) and sleep (Pittsburgh Sleep Quality Index). PWH had cerebrospinal fluid (CSF) sampling for HIV RNA quantification. Global cognition was assessed by T-scores and low cognitive performance by global deficit score ≥0.5. Mixed effects regression models were used to investigate the effects of switch on cerebral function parameters.

Results: Global cognitive performance was 2.57 T-score points lower in PWH than PWoH at baseline (p<0.001), but was not significantly different between groups at follow-up (figure). Rates of low cognitive performance were higher in PWH at baseline (30.1 vs. 11.7%, p<0.001), but not different to PWoH at follow-up (up (8.28 vs. 7.50%, p=1). Mixed effects models showed PWH improved 1.40 points more than PWoH (0.21-2.58, p=0.021). Overall sleep quality improved following switch (OR 0.37, p=0.001), driven mainly by indicators of disturbed sleep. Rates of depressive symptoms (CESD ≥16) worsened (OR 6.53, p=0.016), although baseline differences between PWH and PWoH were present (9.55% vs. 17.1%, p=0.004). 104/113 (92.0%) plasma and 93/94 (98.9%) CSF samples were supressed <50 copies/ml at baseline, and 103/122 (84.4%) and 73/77 (94.8%) at follow up. There was 1 case (1.1%) of CSF HIV RNA escape (CSF HIV RNA > plasma) at baseline and 3 (3.9%) at follow up; 3/4 were at low levels (CSF HIV RNA <200 copies/ml) and 1/4 resolved on repeat sampling without change in ART.

Conclusion: Rates of low cognitive performance were lower than previously reported in this setting, and no different to PWoH once switched to DTG. Observed improvements in cognitive performance and sleep were likely related to switching away from EFV. The increase in depressive symptoms on DTG is inconclusive due to baseline differences, but warrants further investigation. CSF escape was uncommon on both EFV and DTG.
that specific ART combinations rather than individual agents are associated with cognition. Future studies should consider complete drug regimens when assessing the risk of long-term neuropsychiatric complications of ART, with attention to the highlighted drug combinations. The figure, table, or graphic for this abstract has been removed.

555 Phenotyping Risk of Polypharmacy and Cognitive Impairment in ACTG A5322: “HAILO”

Robert Paul1, Kristine M. Erlandson1, Kunling Wu1, Scott L. Letendre1, Jacob Bolzenius1, Katherine Tassiopoulos1, Kyu Choi1, Qing Ma1, Ronald J. Ellis1, Priya Kosana1, Julie Mannarino1, Shelli Farhadian1, for the ACTG A5322 Study Team

1University of Missouri St Louis, St Louis, MO, USA, 2University of Colorado Anschutz Health Medical Campus, Aurora, CO, USA, 3Harvard TH Chan School of Public Health, Boston, MA, USA, 4University of California San Diego, La Jolla, CA, USA, 5University at Buffalo, Buffalo, NY, USA, 6University of California San Diego, San Diego, CA, USA, 7Yale University, New Haven, CT, USA.

Background: Polypharmacy is associated with worse cognitive health among people with HIV (PHW), however explanatory models have not been established. This study leveraged data from a large and well characterized cohort of virally suppressed individuals age 40 and older to identify risk factors that explain the association between polypharmacy and cognitive impairment among PHW.

Methods: Data obtained at enrollment into ACTG A5322 (“HAILO”) were included. Cognitive performance was measured using four tests of verbal learning, attention/psychomotor speed, and fine motor speed and dexterity. Hierarchical density-based spatial clustering, an unsupervised machine learning based algorithm, was used to identify sub-groups based on cognitive performance (Polypharmacy [≤5 ART Medications], hyperpolypharmacy (≥10 medications) as well as demographic, clinical, and psychosocial variables were compared across clusters using multinomial regression, adjusted for multiple comparisons.

Results: Participants were 870 PHW (18.4% female, 52.2% non-White), with a median age of 51. Analyses identified 8 cognitive clusters. There were no differences in average age across clusters. Cluster 1 (33% of the sample) included participants with the best test scores whereas clusters 6, 7, and 8 (collectively 48%) had the worst test scores. Polypharmacy was more common in clusters 6 and 8 compared to cluster 1 (reference group) and hyperpolypharmacy was more common in clusters 7 and 8 compared to cluster 1 (<p<0.05; Table). Participants in clusters 6, 7, and 8 were also more likely to be Black, Hispanic, less educated, and have higher rates of cardiovascular disease, diabetes, hepatitis C, peripheral neuropathy, and substance use compared to participants in cluster 1 (<p<0.05; Table). Participants in clusters 6, 7, and 8 also showed a shorter duration of ART, lower CD4+T-cell count and nadir, and lower CD4/CD8 ratio compared to cluster 1. Use of psychoactive medications did not differ between the clusters. Black or Hispanic women were more likely to have polypharmacy or hyperpolypharmacy (OR=1.4; 95th CI [1.0-2.0]) and cognitive impairment (OR=2.8; 95th CI [1.7-4.6]) compared to any other female. Poor cognitive performance was more common in clusters 7 and 8 compared to cluster 1 (p≤.05). Participants in clusters 6, 7, and 8 were more likely to have polypharmacy or hyperpolypharmacy (OR=1.4; 95th CI [1.0-2.0]) and cognitive impairment (OR=2.8; 95th CI [1.7-4.6]) compared to any other female. Poor cognitive performance was more common in clusters 7 and 8 compared to cluster 1 (p≤.05). Multivariate logistic regression analyses were used to determine associations between genetic polymorphisms and early NPAEs.

Conclusion: Among participants who switched from efavirenz to dolutegravir, early insomnia events were common, but these were not associated with known functional polymorphisms. CYP2B6 slow metaboliser genotype was associated with lower dolutegravir exposure in both arms, but this was only statistically significant in the supplementary dolutegravir arm (β = -1.457 (95% CI -2.467 to -0.44); P = 0.006; Figure 1).

556 Pharmacogenetics of Early Neuropsychiatric Adverse Events After Switching to DTG in Second Line ART

Ying Zhao, Gary Maatens, Rulan Griesel, Graeme A. Meintjes, Phumlwa Sinxadi

University of Cape Town, Cape Town, South Africa

Background: Dolutegravir is associated with neuropsychiatric adverse events (NPAE) in adults failing efavirenz-based antiretroviral therapy and switching to a dolutegravir-based regimen.

Methods: We conducted a pharmacogenetic sub-study of participants enrolled into the ARTIST clinical trial, who were switched from tenofovir-emtricitabine-efavirenz to tenofovir-lamividine-dolutegravir and randomised to supplementary dolutegravir 50 mg dose or placebo for the first 2 weeks. Primary outcome was change in sleep quality from baseline to week 2. NPAs were assessed by questionnaires and neuropsychological testing before and after the switch. Plasma dolutegravir trough concentrations were collected at week 2. We genotyped polymorphisms relevant to efavirenz disposition [CYP2B6 rs3740274 G → T, rs2839949 T → C, and rs4803419 C → T], and CYP2A6 rs2839433 A → C), dolutegravir disposition (UGT1A1 rs887829 C → T), and dolutegravir toxicity (SLC22A2 rs61091 C → A). Multivariate logistic regression analyses were used to determine associations between genetic polymorphisms and early NPAEs.

Results: 128 participants were evaluable for genetic analyses. The median age was 38 years (IQR 32~45), 68% female, median duration on ART was 85 months (IQR 50~119), and median baseline HIV-1 RNA was 4.0 log. copies/mL (IQR 3.5~4.7). Insomnia events occurred in 17 (13%) participants. There were no statistically significant associations between genetic polymorphisms and worsening sleep quality. UGT1A1 rs887829 homozgous TT genotype was associated with higher dolutegravir exposure (β = 0.841 (95% CI -0.30 to 1.711), P = 0.058). CYP2B6 slow efavirenz metaboliser genotype was associated with lower dolutegravir exposure in both arms, but this was only statistically significant in the supplementary dolutegravir arm (β = -1.457 (95% CI -2.467 to -0.447), P = 0.006; Figure 1).

Conclusion: Among patients who switched from efavirenz to dolutegravir, early insomnia events were common, but these were not associated with known functional polymorphisms. CYP2B6 slow metaboliser genotype was associated with lower dolutegravir exposure, reflecting prolonged efavirenz induction effect.
DTG, average concentrations of 196 ng/g were recorded compared to 34 ng/g and 45 ng/g for single or two IM injections of NDTG, respectively. MRI scanning of live dams was performed to acquire T1 maps of the embryo brain to assess oxidative stress. Significantly lower T1 values were noted in daily oral DTG-treated mice, whereas comparative T1 values were noted between control and NDTG-treated mice, indicating prevention of DTG-induced oxidative stress when delivered as NDTG. Proteomic profiling of embryo brain tissues demonstrated reductions in oxidative stress, mitochondrial impairments, and amelioration of impaired neurogenesis and synaptogenesis in NDTG-treated group.

**Conclusion:** This work suggests that long-acting drug delivery can prevent DTG-linked neurodevelopmental deficits by limiting drug exposure to the embryo brain.

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558 A New Measure of ART Activity in CSF and Association With Persistence and Cognitive Function


**University of Nebraska Medical Center, Omaha, NE, USA**; **Harvard TH Chan School of Public Health, Boston, MA, USA**; **Yale University, New Haven, CT, USA**; **The Johns Hopkins Hospital, Baltimore, MD, USA**; **University of Pittsburgh, Pittsburgh, PA, USA**; **University of North Carolina at Chapel Hill, Chapel Hill, NC, USA**; **Massachusetts General Hospital, Boston, MA, USA**

**Background:** ACTG A5321, a prospective study of HIV-1 reservoirs among persons with HIV on antiretroviral therapy (ART), previously showed detection of HIV DNA in cells from cerebrospinal fluid (CSF) was associated with poorer global cognitive function. We conducted a cross-sectional analysis of antiretroviral (ARV) pharmacokinetics (PK) in CSF and investigated relationships among a novel measure of ART regimen activity and HIV persistence in CSF and cognitive function.

**Methods:** Participants were on ART for a median of 8.1 years with sustained plasma HIV suppression at time of lumbar puncture (LP). CSF ARV concentrations, cell-associated HIV DNA and inflammatory biomarkers were measured; a neuropsychological test battery (outcome=global deficit score, GDS) was administered just prior to or at LP. ARV levels were quantified by LC/MS/MS. Population PK modeling was used to estimate CSF drug exposure. CSF inhibitory quotients (IQ) were calculated for each ARV in a regimen as ratio of inhibitory quotients (IQ) were calculated for each ARV in a regimen as ratio of CSF IQs of all drugs in each participant’s ARV regimen was calculated (ART-IQ-GeoM). Statistical analyses evaluated associations among the ART-IQ-GeoM and CSF HIV DNA, biomarkers and GDS. CSF IQs of all drugs in each participant’s ARV regimen was calculated (ART-IQ-GeoM). Statistical analyses evaluated associations among the ART-IQ-GeoM and CSF HIV DNA, biomarkers and GDS.

**Results:** CSF ARV levels were measured in 44 chronic-treated participants on TDF/FTC-based ART: 43 (98%) male sex at birth; 36 (97% of 37) male gender; median CD4 count, 642 cells/µL; 43 (98%) with plasma HIV RNA <40 copies/mL. The geometric mean of CSF IQs of all drugs in each participant’s ARV regimen was calculated (ART-IQ-GeoM). Statistical analyses evaluated associations among the ART-IQ-GeoM and CSF HIV DNA, biomarkers and GDS.

**Conclusion:** The ART IQ metric is a new approach to assess ART regimen activity. Higher ART-IQ-GeoM was associated with a lack of detection of CSF HIV DNA and better global cognitive function. These findings suggest ART regimen activity affects HIV persistence in CSF. This tool provides a basis for further investigations of relationships between regimen activity and biomarkers of HIV persistence in the CSF and other viral reservoirs.

559 The Association Between Prior SARS-CoV-2 Infection and Incidence of Stroke


**Hamad Medical Corporation, Doha, Qatar; Weill Cornell Medicine College in Qatar, Doha, Qatar**

**Background:** Individuals with COVID-19 have an increased incidence of several comorbid conditions including diabetes and acute myocardial infarction. The association of COVID-19 infection with stroke is controversial, with some studies demonstrating a higher risk and other studies showing no association or even a protective effect. We sought to determine the association between COVID-19 infection and subsequent incidence of stroke at a national level in Qatar.

**Methods:** We used the Qatar Stroke Database to identify individuals who were admitted with acute ischemic or hemorrhagic stroke to the tertiary care referral hospital in Qatar, which accounts for 98% of all acute stroke admissions in Qatar. For the current study, we included individuals admitted with acute stroke between March 1, 2020 and April 11, 2023. We linked the Qatar Stroke Database to the Qatar National COVID-19 database to retrieve the information on COVID-19 testing and vaccination. This database contains all records of RT-PCR and medically-supervised antigen testing and vaccination in the State of Qatar. Eligible individuals with stroke diagnosis were exactly matched 1:1 on 10-year age group, sex, nationality, type of comorbidity, and number of vaccine doses received, to eligible controls who tested SARS-CoV-2-negative during the same week of the stroke diagnosis. We utilized a case–control design to determine the association of COVID-19 diagnosis with acute stroke. Adjusted odds ratios and corresponding 95% confidence intervals were calculated for the entire study population and subgroups by age, nationality, and prior infection variant period (infection in the pre-omicron era, omicron era, or in both eras).

**Results:** A total of 1,640 matched pairs were analyzed. Median age was 49 years, 85% were male, 11% were Qatari nationals, 58% had no comorbidities, and 48% were unvaccinated at the time of first stroke diagnosis. Any prior infection was associated with a lower risk of stroke (aOR 0.48, 95% CI 0.40,0.58). The protective association was consistent across older age groups, among unvaccinated and vaccinated, the infection era (pre-omicron or omicron eras) and regardless of the time from infection. (Table)

**Conclusion:** Prior COVID-19 infection is associated with a lower risk of stroke. This association is independent of age, vaccination status, infection era by protective effect. We sought to determine the association between COVID-19 infection and subsequent incidence of stroke at a national level in Qatar.

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560 Focal Cerebral Hypoperfusion in Individuals With Cognitive Impairment After COVID-19

Lindsay S. McAlpine, Allison Nelson, Jennifer Chiarella, Robert Fulbright, Shelli Farhadian, Maolin Qiu, Todd Constable, Serupa Suditch.

**Yale University, New Haven, CT, USA**

**Background:** Cognitive impairment is a common symptom of neuropsychiatric post-acute sequelae of COVID-19 (N-PASC), characterized by neuropsychological deficits including impaired executive functioning, processing speed, motor speed, attention, recall, and verbal fluency. Little is known about the underlying mechanism of cognitive N-PASC. We report preliminary analyses of noninvasive MRI measurements of brain perfusion in individuals with and without N-PASC.

**Methods:** Participants with cognitive N-PASC (self-reported symptoms of cognitive impairment >3 months after COVID-19) referred to a NeuroCOVID Clinic and controls with prior COVID without PASC underwent an MRI protocol, which included Arterial Spin Labeling (ASL) to assess perfusion (Cerebral Blood Flow; CBF). All imaging was performed on a Siemens 3T MRI scanner. A standard
3D ASL sequence was used (TA: 4.59, voxel: 1.5x1.5x3.0 mm3, RelSNR: 1.00, TR: 4600ms, TE: 16.18ms). Post-processing was completed using MATLAB and the Harvard-Oxford atlas to generate CBF for 48 cortical and 21 subcortical regions of interest (ROIs). Group comparisons used non-parametric statistics.

Results: 14 participants with cognitive N-PASC (median age 43 [IQR 37–55]), 79% female, median 450 days after COVID-19 symptom onset (IQR 354–664) and 6 controls (median age 34 [30–40], 67% female) underwent MRI. The groups did not differ in age, gender, race, or cardiovascular risk factors, which were low in prevalence. CBF was lower in N-PASC compared to controls (C) in the right supplementary motor area (N-PASC: median of 22.5 mL/g/min and C: 27.7 mL/g/min; p = 0.025), a trend of hypoperfusion that did not reach significance was identified in three other ROIs in the right hemisphere: the frontal pole (N-PASC: 23.5 mL/g/min and C: 30.4 mL/g/min; p = 0.06), middle frontal gyrus (N-PASC: 24.8 mL/g/min and C: 31.3 mL/g/min; p = 0.06), and post-central gyrus (N-PASC: 22.5 mL/g/min and C: 27.7 mL/g/min; p = 0.06). There was no difference in CBF between groups in the remaining ROIs.

Conclusion: We report preliminary evidence of focal hypoperfusion in the right frontal lobe and a trend of hypoperfusion in the right parietal lobe in individuals with N-PASC. These findings suggest that altered perfusion in the non-dominant hemisphere may play a role in cognitive N-PASC symptoms, possibly by affecting motor speed and motor control of speech. We look forward to collecting additional data to investigate the mechanism of decreased cortical perfusion in cognitive N-PASC.

651 CSF Biomarker Evidence of Synaptic Dysfunction in Acute, but Not Post-Acute COVID-19

Arvid Edén, Johanna Nilsson, Anna Grahn, Nelly Kanberg, Erika Stentoft, Daniel Brennert, Aylin Yilmaz, Marie Studahl, Staffan Nilsson, Michael Schöll, Iris Bosch, Kaj Blennow, Ann Brinkmalm, Henrik Zetterberg, Magnus Gišlén Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Background: CNS immune activation and neurocognitive symptoms are common in severe COVID-19, but mechanisms of persisting CNS dysfunction in post-COVID-19 conditions (PCC; “long covid”) are unclear. We used a panel of CSF markers, several of whom have been implicated in Alzheimer’s disease (AD) and other neurodegenerative diseases to investigate synaptic and lysosomal dysfunction in acute and post-acute COVID-19.

Methods: Lumbar punctures were performed on 76 (49 male) patients and 20 (6 male) healthy controls from longitudinal studies, sampled during acute (46), ≥3 (39 [31 PCC]) and/or ≥12 (25 [19 PCC]) months after COVID-19. PCC symptoms at follow-up were evaluated by interview and self-report. The 37-marker CSF panel included catecholamines, calciotryptins, contactins, granins, glutamate receptor 4 (GRIIA4), VGF, APP, neurogranin, syntaptins, LAMPS, beta-hexosaminidase subunit beta (HEXB), GM2A, dipeptidyl peptidase 2 (DPP-2), NCA22, NSF, synapsin-1, CAMK2A, Thy-1, VAMP2, AP2B1, complexins, synucleins, GDI-1, neuronal pentraxins, PEBP-1, and members of the 14-3-3 protein family. A micro-high performance liquid chromatography mass spectrometry system (6495 Triple Quadrapole LC/MS system, Agilent Technologies) equipped with a HyperVial Gold reversed-phase C18 column (dim: = 100×2.1 mm, particle size: = 1.9 μm, Thermo Fisher Scientific) was used for quantitation. Group comparisons of biomarker concentrations were analyzed by Kruskal-Wallis size=1.9 μm, Thermo Fisher Scientific) was used for quantitation. Group comparisons of biomarker concentrations were analyzed by Kruskal-Wallis

Conclusion: Several markers previously associated with neurodegenerative and psychiatric diseases were significantly altered during acute COVID-19. Notably, neuronal pentraxin-2 (a sensitive marker of cognitive decline in AD) remained unaffected. Importantly, no biomarker evidence of persisting CNS pathology was seen at follow-up either between patients and controls, or between patients with or without PCC indicating that PCC is not associated with progressive postinfectious neurodegeneration.

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562 SARS-CoV-2 Nucleocapsid Antigen is Not Detected in the CSF During Long COVID

Shelli Farhadian, Allison Grubman, Lindsay S. McAlpine, Bibhuprasad Das1, Jennifer Chiarella1, Benjamin Orlnick2, Hailey Reisert1, Allison Nelson1, Priya Kosa1a, Meenakshi Khare3, Serena Spudich1

1Yale University, New Haven, CT, USA; 2Quanterra Corporation, Lexington, MA, USA

Background: SARS-CoV-2 viral protein persistence has been suggested as a possible cause of post acute sequelae of COVID-19. We previously showed that some individuals with neuropsychiatric symptoms after COVID-19 had persistent SARS-CoV-2 nucleocapsid antibodies in the cerebrospinal fluid (CSF) several months after COVID-19. However, it is unknown whether this is driven by persistence of SARS-CoV-2 antigen in the CSF.

Methods: CSF and plasma was collected from participants in four groups: acute COVID (hospitalized with acute-infection, n=5); post-COVID with neuropsychiatric symptoms (Neuro-PASC, n=31); post-COVID asymptomatic (n=8), and pre-pandemic never-COVID controls (n=20). Tissue samples were measured for SARS-CoV-2 Nucleocapsid (N) antigen levels via a Single Molecular Array (Simoa) immunoassay, a paramagnetic microbead-based sandwich ELISA (Quanterix, Billerica, MA, USA). Samples were run in duplicate, and the results were averaged. CSF was run undiluted and plasma samples were diluted 1:4 per manufacturer protocol, with results corrected for dilutions.

Results: Sixty-four participants’ samples were analyzed: sixty-one paired CSF and plasma samples and three CSF samples. Acute COVID participants were sampled a median of 7 days (range 1–34) after symptom onset. Neuro-PASC and post-COVID asymptomatic participants were sampled a median of 381 days (range 81–1157) after acute infection. SARS-CoV-2 N-antigen was not detected in any of the post-COVID (Neuro-PASC or asymptomatic) or pre-pandemic participants, in CSF or plasma. SARS-CoV-2 N-antigen was detected in the CSF of three participants, all of whom were from the acute COVID cohort. Of these three participants, one had antigen present in the CSF sample (1.40 pg/mL), one had antigen present in the plasma sample (29.9 pg/mL), and one had antigen present in both the CSF and plasma samples (12.6 pg/mL and 785 pg/mL, respectively).

Conclusion: We found SARS-CoV-2 N-antigen is undetectable in the plasma and CSF of post-COVID participants, those with Neuro-PASC and those without PASC. Neuro-PASC is unlikely to be caused by persistent SARS-CoV-2 N-antigen in the CNS.
563 Gut Microbiome Dysbiosis and Lower Abundance of Butyrate Producing Bacteria in Neurologic PASC
Lawrence Purpura, Lingsheng Wen, Heekuk Park, Ga Young Seo, Jayesh Shah, Amanda Castillo, Anne-Catin Uhrmann, Michael T. Yin
Columbia University Medical Center, New York, NY, USA

Background: Neurologic post-acute sequelae of COVID-19 (PASC) affects a growing population of individuals, with symptoms including myalgic encephalomyelitis chronic fatigue syndrome (ME-CFS), cognitive dysfunction, dysautonomia, and neuropathy. Gut microbiome dysbiosis has been reported in the early convalescent period after SARS-COV-2 infection (<1 year) in hospitalized patients, with lower alpha diversity and decreased abundance of butyrate-producing species. Butyrate has a protective effect in the gut by supporting the mucosal barrier and has systemic anti-inflammatory and immunomodulatory effects. Notably, in long-term ME-CFS patients (symptoms >10 years), metabolic dysfunction persists despite recovery of relative abundance of bacterial species. To date, the longitudinal role of gut dysbiosis in neurologic PASC remains unknown, especially in patients with mild acute COVID-19.

Methods: The COVID-19 Persistant and Immunology Cohort (C-PIC) is an observational cohort study with over 650 participants, with and without PASC. Rectal swabs or stool samples are collected at 3-6 month intervals, with matched clinical metadata. Neurologic PASC is defined as self-reported fatigue, cognitive dysfunction, or dysautonomia. DNA was extracted using the Zymo MagBead DNA/RNA kit and the MiSeq platform was used to sequence the V3/V4 region of the bacterial16s RNA gene. DESeq2 was used to assess species-level differential abundance testing and alpha diversity was measured using Chao scores for richness. Differences between participants with and without neurologic PASC were compared in C-PIC participants with mild acute COVID-19 not requiring hospitalization.

Results: 151 C-PIC participants with mild acute COVID-19 were included in the analysis. Fecal samples collected at study enrollment demonstrated lower relative abundance of butyrate-producing species (Faecalibacterium prausnitzii, Eubacterium spp, Bacteroides spp) in participants reporting neurologic PASC (p<0.05). We also report a trend toward increasing alpha diversity (chaos) over time across 371 longitudinal samples (figure).

Conclusion: Our findings of decreased abundance of butyrate-producing bacterial species in patients reporting neurologic PASC and a trend towards improving alpha diversity after recovery from SARS-COV-2 align with late convalescent findings in ME-CFS. These data provide support for the role of gut microbiome in neurologic PASC. Further research is needed to identify gut microbiome targets for diagnostics and therapeutics.

564 Brain White Matter Hyperintensity Accumulation in an Acute HIV Cohort Over 2 Years
Kathryn Holroyd, Jacob Bolzenius1, Carlo P. Sacdalan3, Netsiri Dummrongpisutikul4, Somchui Sripriamsan1, Patharaya Promsri1, Sandhya Vasam1, Lydie Trautmann2, Matthew J. Mimiaga1, Kathleen Weber1, Parinya Wheeler2, Leah H. Rubim1, Felicia C. Chow1
1University of California San Francisco, San Francisco, CA, USA, 2University of Miami, Miami, FL, USA, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 4Emory University, Atlanta, GA, USA, 5The Johns Hopkins University, Baltimore, MD, USA, 6State University of New York Downstate Medical Center Downstate Medical Center, Brooklyn, NY, USA, 7University of Washington, Seattle, WA, USA, 8University of Pittsburgh, Pittsburgh, PA, USA, 9University of California Los Angeles, Los Angeles, CA, USA, 10Hektoen Institute of Medicine, Chicago, IL, USA, 11University of Alabama at Birmingham, Birmingham, AL, USA, 12The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Higher cardiovascular risk is associated with poorer cognitive health, including in people with HIV (PWH). We examined whether HIV modifies the association between the Atherosclerotic Cardiovascular Disease (ASCVD) Risk Estimator and subsequent cognition in the Multicenter AIDS Cohort Study (MACS) and Women’s Intergeneracy HIV Study (WHIS).

Methods: Participants followed in the MACS (N=1773 men, 2005 to 2019) and WHIS (N=1264 women, 2009 to 2019) who had available CVD risk data and underwent neuropsychological (NP) testing at least 1 year after calculated ASCVD risk score were included. Demographically-adjusted T-scores for the cognitive tests that overlapped between the two cohorts were averaged as a global NP score and in 4 domains (motor function: Dominant/Non-dominant Grooved Pegboard; executive function: Trail-Making Part B, Stroop Color-Word; attention: Trail-Making Part A, Stroop Word-Reading; processing speed:...
Cumulative Exposure to CVD Risk Factors More Adversely Affects Cognition in Women With & Without HIV

Abel C. Obosi1, Yifei Ma1, Maria L. Alcaded2, Cecile B. Lahiri1, Monica M. Diaz1, Gysypammer D’Souza3, Deborah Gustafson1, Sable Kassaye2, Matthew J. Mimiaga3, Yifei Ma1, Robert Paul1, Leah H. Rubin1, Adesola Ogundiyi1, Rabafemi Taiwo1, Felicia C. Choe1, Hannah K. Fandl1, Elizabeth Connick1, Seble Kassaye2, Kathleen Weber2,3, Deborah Gustafson1, Robert Paul1, Leah H. Rubin1, Adesola Ogundiyi1, Rabafemi Taiwo1, Felicia C. Choe1

Background: The adverse effect of cardiovascular disease (CVD) risk factors on cognitive health may be greater for women than men. We evaluated if sex and HIV modify the effect of CVD risk on cognition in the Women’s Interagency CVD Study (WHS) and the Multicenter AIDS Cohort Study (MACS).

Methods: People living with HIV (PWH) and without HIV who underwent neuropsychological testing at least once beginning in 2005 in the MACS and in 2009 in the WHS were eligible. We examined performance on overlapping tests (TrailMaking A and B, Symbol Digit Modalities, Stroop Color Naming). We constructed random-effects panel linear regression models to estimate the overall, HIV, and sex-stratified associations of ASCVD risk with subsequent cognitive function (NP testing performed median 2 years later, range 1-14 years) in the combined and separate cohorts.

Results: In the combined cohort (mean age 46 years, 39% women), median ASCVD risk score was 3.4% (IQR 1.2-7.6%). ASCVD risk score was higher in people without HIV (3.8%, IQR 1.3-8.6%) than in PWH (3.1%, IQR 1.1-6.8%; p<0.001). Higher ASCVD risk score (per IQR for all results) predicted lower subsequent global cognition (β -0.41, SE 0.04, p<0.001). HIV did not modify the association between ASCVD risk and cognitive function (interaction p=0.465). In men, no difference in the association between ASCVD risk and subsequent cognition (global or in domains) was observed by HIV. In women, higher ASCVD risk significantly predicted lower subsequent global cognition (β -0.20, SE 0.02, p=0.016) and motor function (β -0.06, SE 0.05, p=0.009) in women with HIV but not in women without HIV (Table).

Conclusion: The ASCVD risk score predicted subsequent cognitive function in PWH and people without HIV, although the magnitude of these associations was modest overall and particularly among women. These findings underscore the complex relationship between HIV and ASCVD risk on cognition. Future studies should examine the cause of these observed differences between women and men.

Table: Association between ASCVD risk score and subsequent cognitive function

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<th>Model</th>
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<th>Motor Function</th>
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567 Circulating Endothelial Microvesicles With HIV-1 Promote Cerebral Endothelial Cell Stroke Profile

Auburn R. Berry1, Samuel Ruzzen1, Kendra Wexgorsen, Emily L. Ostrander1, Hannah L. Cardenas1, Hannah K. Fandl1, Jared J. Greiner, Vinicus P. Garcia1, Elizabeth Connick1, Kathleen Weber2,3, Deborah Gustafson1

Background: The incidence of ischemic stroke in adults living with HIV (ALWH) is three times higher than healthy adults. Circulating endothelial cell-derived microvesicles (EMVs) have been linked to cerebrovascular events. We have previously reported that EMVs isolated from ALWH receiving antiretroviral therapy (ART) impair brain endothelial cell nitric oxide (NO) production and fibrinolytic capacity, central etiologic mechanisms in the pathogenesis of ischemic stroke. However, it is unknown whether the pathologic EMV phenotype is a consequence of HIV per se or ART. The experimental aim of this study was to determine if the effect of EMVs isolated from treatment naive ALWH on brain endothelial cell nitric oxide production and fibrinolytic capacity.

Methods: Circulating EMVs (CD 144-PE) were isolated (flow cytometry) from 16 young and middle-aged men (age range: 21-43 yr); 8 healthy (age 33±3 yr; BMI: 26±2.1 kg/m²; BP: 113±7/72±22 mmHg) and 8 treatment naive ALWH (10M/2F; 36±22 yr; 25±3±1.5 kg/m²; BP: 117±76±3/3 mmHg; viral load: 5525 copies/mL). All men were free of overt cardiometabolic disease and not taking any medication. Human cerebral microvascular endothelial cells (HCMECs) were cultured and separately treated with EMVs from each subject.

Results: Circulating EMVs were significantly higher in the treatment naïve ALWH compared with healthy adults (123±5±3 EMVs/mL). Although total endothelial nitric oxide synthase (eNOS) expression was not significantly altered (60±4±2 vs 65±3±1 AU); active eNOS (pSer1177) (19±0.8±0 vs 26±6±1.5 AU) and, in turn, NO production (5.7±0.2 vs 6.7±0.3 μmol/L) was lower (P<0.05) in cells treated with EMVs from treatment naive ALWH vs EMVs from healthy adults. HIV-associated EMVs also significantly reduced tissue-type plasminogen activator (t-PA) (25±5±2 vs 34±7±1 AU) and increased plasminogen activator inhibitor (PAI)-1 (146±0.4±5 vs 110±6±3 AU) protein expression in HCMECs. The t-PA:PAI-1 intracellular protein ratio (5.9±0.3 vs 3.3±0.1 AU; P<0.05) was higher in HIV-1 EMV treated cells, indicative of decreased fibrinolytic capacity.

Conclusion: HIV-1, independent of traditional risk factors and ART, is associated with a pathologic circulating EMV phenotype. Reduced NO bioavailability and impaired fibrinolytic capacity in brain endothelial cells heighten the risk.
568  Vascular Inflammation in Neuropsychiatric Post-Acute Sequelae of COVID-19
Lindsay S. McAlpine, Hailey Reisert, Bibhuprasad Das, Allison Nelson, Jennifer Chiarella, Sheill Farhadian, Serena Spudich
Yale University, New Haven, CT, USA
Background: Neuropsychiatric post-acute sequelae of COVID-19 (N-PASC) include cognitive impairment, mood changes, headache, and neuropathy. Biomarkers of endothelial and platelet dysfunction are elevated in patients with acute COVID-19, but it is unknown if this persists in individuals with N-PASC. We investigated for vascular inflammation in N-PASC and controls.

Methods: Participants with N-PASC (ongoing neuropsychiatric symptoms >3 months after COVID-19) and controls underwent cross sectional clinical assessment and blood collection. Plasma samples were tested via multiplex bead-based ELISA for the following analytes: a-2 macroglobulin, α1-acid glycoprotein (AGP), C-reactive protein (CRP), Fetuin A36, haptoglobin, L-selectin, platelet factor 4 (PF4), and serum amyloid protein (SAP) A (Eve Technologies). Non-parametric multiple Mann-Whitney testing was used with False Discovery Rate adjustment made to address multiple comparisons.

Results: The N-PASC (N=40) and control (C; n=16) groups were similar in age (N: 45 years, C: 40 years, P=0.15), gender (N: 73% female, C: 69% female, P=0.76), race (N: 20% non-white, C: 37% non-white, P=0.19) and cardiovascular risk factors (diabetes, smoking, hypertension, obesity, and cardiac disease, P>0.05). The groups had similar time from acute COVID-19 to study visit (N:325 days, C:418, P=0.95). N-PASC symptoms included cognitive issues (72%), new or worsening anxiety or depression (67%), and headache (61%). Five markers were elevated in N-PASC: a-2 macroglobulin (N: 994,143 ng/mL, C: 749,109, P<0.04), CRP (N: 8,851,400 pg/mL, C: 3,625,000, P<0.01), haptoglobin (N: 194,735 ng/mL, C: 99,319, P<0.046), L-selectin (N: 808,346 pg/mL, C: 670,940, P<0.03), and SAP (N: 6,252,000 pg/mL, C: 3,186,650, P=0.0003) (Figure). Fetuin A36 was reduced (N: 132,476 ng/mL, C: 207,355, P<0.05). There were no differences in the other biomarkers tested.

Conclusion: We report key differences in vascular inflammatory plasma biomarkers in individuals with N-PASC, including elevations in plasma proteins that indicate ongoing systemic inflammation (CRP, haptoglobin, SAP), endothelial dysfunction (a-2 macroglobulin), and atherosclerosis (L-selectin, fetuin A36, SAP). These findings suggest the N-PASC population may be at risk of persistent vascular inflammation and/or atherosclerosis. Further studies should longitudinally investigate endothelial inflammation and atherosclerosis in individuals with N-PASC.

569  Verbal Learning and Memory in Well-Controlled HIV Is Similar to People Without HIV in Uganda
Noeline Nakasuja1, Leah H. Rubin1, Deanna Sayler1, Aggrey Anok1, Stephen Tomusange1, Maria J. Wawer2, Jacob Bolzenius3, Deanna Saylor3, Gertrude Nakigozi3
Makerere University College of Health Sciences, Kampala, Uganda, 1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2University of Missouri St Louis, St Louis, MO, USA
Background: Cognitive impairment is common among people with HIV (PWH) in the United States especially in verbal learning and memory. Notably, cardiometabolic risk factors and complications and substance misuse have been associated with cognition. In Rakai, Uganda, these complications are less common in middle-aged PWH. We examined the degree to which HIV-serostatus affects learning and memory in PWH and people without HIV (PWoH) in Rakai, Uganda.

Methods: Participants enrolled through the Rakai Community Cohort Study (350 PWH; 250 PWoH) were administered a neuropsychological assessment which included the WHO—Auditory Verbal Learning Test, a list-learning task where participants are asked to recall as many words as possible across five learning trials. The test was administered in Luganda by trained research staff. For delay recall, the participant is asked to recall the original list after a 20-minute delay. Primary outcome measures include total learning across trials and total correct words recalled on the delay recall trial. Demographically-adjusted (age, sex, education) z-scores were established using data from PWoH. Impairment was defined as one standard deviation below PWoH (z-score <1). The two groups were compared using a Chi-Square Test.

Results: PWH were demographically-similar to PWoH in terms of age (44 vs. 43 years, P=.53), sex (48% vs. 54% male, P=.31), and education (6 vs. 5.8 years, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31). Cardiovascular and metabolic comorbidities were low in each group, including obesity (9% vs. 12%, P=.89), history of diabetes (6% vs. 3%, P=.31).

Conclusion: PWH on long term ART with relatively few comorbidities in Uganda demonstrate a low prevalence of verbal learning or memory impairment which is similar to that of PWoH. Possibly, the level of health care for PWH in Uganda may serve as a protective factor for HIV cognitive decline however, as the Rakai cohort ages it may be at risk for developing cognitive impairment and the emergence of age-related health comorbidities.
571  Plasma Inflammatory Biomarkers Link to Worse Cognition Among Africans Living With HIV

Samuel Wilson, Andjeilia Milicic, Rither Langat, Winnie Rehema, Gloria David, Nikichemire Harrison, Hannah Kibuuka, Hendrik Streck, Alifana Eshber, Shireen Javandel, Isabel Allen, Lisowonna Ndhlouv, Julie Ake, Victor Valcour, for AFRICOS

Thomas Jefferson University, Philadelphia, PA, USA, University of California San Francisco, San Francisco, CA, USA, US Military HIV Research Program, Bethesda, MD, USA, IMF Medical Research International, Mbande, United Republic of Tanzania, Walter Reed Project—Kisumu, Kisumu, Kenya, Visiting Faculty—Nigeria, Abuja, Nigeria, Makerere University Walter Reed Project, Kampala, Uganda, University of Bonn, Bonn, Germany, Weill Cornell Medicine, New York, NY, USA

Background: Sub-Saharan Africa (SSA) accounts for nearly two-thirds of global HIV infections. Despite access to therapy, comorbidities in the region remain prevalent, including cognitive impairment (CI). The neuropathogenesis of CI among treated people with HIV is thought to be driven by inflammation, particularly linked to monocytes.

Methods: We characterized the relationship between inflammatory plasma biomarkers (CXCL10, CCL2, sCD163, and sCD25) and cognitive performance in a Sub-Saharan African cohort of people living with HIV and people without HIV at sites in Kenya, Nigeria, Tanzania, and Uganda (AFRICOS). All assessments were completed at the time of enrollment into the AFRICOS cohort.

Neuropsychological assessments included the WHO/NIMH Auditory Verbal Learning Task, Trail Making A and B, Stroop Test, Boston Naming Test, Visual Object and Space Perception Battery, Digit Symbol, 15-Letter Substitution, Grooved Pegboard, and the Test of Everyday Attention. Cognitive assessments were completed at the time of enrollment into the AFRICOS cohort. All participants had CD4 counts performed at the time of enrollment.

Results: In all, 473 (17%) were living without HIV, 1393 (51%) were living with HIV and suppressed plasma viremia, whereas 871 (32%) had unsuppressed plasma viremia (>1000 copies/mL). Compared to controls, the group of people living with HIV who had older (p<0.001) and less literate (p=0.013). We found inverse relationships (see Table) between plasma biomarkers and cognitive performance on all measures except sCD163 in the people living with HIV, particularly in the suppressed group. Inflammation was not associated with cognitive performance among controls. The Grooved Pegboard test appeared to have the strongest associations between inflammatory markers and worse cognitive performance.

Conclusion: In the sub-Saharan African context, chronic inflammation among people living with HIV is linked to worse cognitive performance. This association persists even in those with suppressed plasma viral load. *First and second authors contributed equally

![Table showing correlations between inflammatory biomarkers and cognitive performance](image)

572  Improving Diagnosis of Central Nervous System Infections in High-HIV Prevalence African Settings

James Milburn, Taddy Nwambara, Rachita Suresh, Kebatshabile Ngqoni, Tavengwa Munyenyi, F. Kathryn Boyd, Lenon Gerwanna, Tiny Mazhani, Ronan Doyle, Katharina kraenzel, Madisa Mine, Margaret Mokokamane, Gift Ngwende, Chiratidzo Ndhlou, Joseph N. Jarvis


Background: Central nervous system infections (CNSI) account for approximately 30% of early mortality in ART programmes. The epidemiology of CNSI in high-HIV-prevalence African settings is poorly understood making presumptive diagnosis and empiric treatment challenging. Patients with CNSI who do not receive a diagnosis have mortality rates of up to 40% at 10 weeks indicating the presence of serious underlying pathology that needs appropriate diagnosis to guide effective treatment.

Methods: Enhanced diagnostic packages were introduced into routine CSF analysis at Princess Maria Hospital, Gaborone, Botswana in June 2021 and Parenyatwa Hospital, Harare, Zimbabwe in October 2022. BioFIRE FilmArray-Meningitis/Encephalitis (FilmArray-ME) & Xpert MTB/RIF Ultra were performed on all samples and results returned to clinicians in real-time. Retrospective analysis was performed on stored samples with Toxoplasma gondii PCR & rapid plasma reagin, Treponema pallidum particle agglutination assay, and metagenomic sequencing.

Results: Sequential CSF samples from 465 people living with HIV with suspected CNSI underwent analysis with FilmArray-ME and 454 had analysis with Xpert MTB/RIF Ultra. The median age of study participants was 40 (IQR 34–48), 54.4% were male, and the median CD4 count was 141 cells/µL (IQR 43–323), 49.0% (220/465) were established on ART the remainder were either ART naive or had cycled out of treatment services. Through routine CSF analysis alone there were 99 microbiologically confirmed CNSI; with the addition of enhanced diagnostics this increased to 155 (Figure 1), a relative increase of 57%. Detection of Mycobacterium tuberculosis, T. gondii and viral pathogens was only achieved through the addition of enhanced diagnostics. Cryptococcal meningitis was the most commonly diagnosed CNSI in 97/465 patients with 92/97 diagnoses made through cryptococcal antigen (CrAg) testing or India ink, figure 1. Restricting analysis to CrAg negative patients, only 11 CNSI diagnoses were made through routine analysis compared to 62 diagnoses made with the addition of enhanced diagnostics, a relative increase of 464%.

Conclusion: Enhanced diagnostic platforms substantially increased diagnostic yield in patients with suspected CNSI in Botswana and Zimbabwe. There was significant variation between population groups suggesting targeted use may be possible. The most clinically and cost-effective diagnostic algorithms need to be defined.

![Figure 1: Potential CNSI pathogens detected through routine and enhanced testing at Princess Maria Hospital, Gaborone and Parenyatwa Hospital, Harare](image)

573  Mental Health Phenotypes of Well-Controlled HIV in Uganda

Leah H. Rubin, Kyu Cho, Jacob Bolzenius, Julie Manninen, Aggrey Anok, Stephen Tomusange, Raha M. Dastgheyb, Maria J. Wawer, Allahna Esber, Noeline Nakasujja, Gertrude Nakigaz, Robert Paul

1. Johns Hopkins University, Baltimore, MD, USA, 2. University of Missouri St Louis, St Louis, MO, USA, 3. Rakai Health Sciences Program, Kalisizo, Uganda, 4. Makerere University, Kampala, Uganda

Background: HIV and mental health (MH) disorders, particularly depression, anxiety, and post-traumatic stress disorder (PTSD), are among the top 10 causes of disability among people with HIV (PWH) in Uganda. Most studies of PWH have focused on MH disorders as unidimensional constructs. However, the phenotypic expression and clinical course of MH conditions among PWH in Uganda and worldwide are heterogeneous. Accordingly, there has been a shift towards identifying MH phenotypes using data driven methods capable of identifying novel insights into mechanisms of divergent MH phenotypes among PWH. We leverage the analytic strengths of machine learning combined with inferential methods to identify novel MH phenotypes among PWH and the underlying explanatory features, with a particular interest in early life stress (ELS) as a determinant of MH phenotypes.

Methods: 277 PWH (46% female, median age=44; 93% undetectable viral load [<50 copies/mL]) were included in the analyses. Participants were enrolled in an observational community-based cohort residing in the Rakai region of Uganda. Participants completed the Patient Health Questionnaire (PHQ-9), Beck Anxiety Inventory (BAI), and the PTSD Checklist-Civilian (PCL-C). Hierarchical clustering was used to identify MH subtypes using total symptom scores on
the questionnaires. Inferential statistics (with false discovery rate) compared demographic and clinical factors between clusters (e.g., ELS).

**Results:** We identified four MH phenotypes (Fig. 1). Cluster 1 (n=76; PTSD phenotype) endorsed clinically significant PTSD symptoms, with an average PCL-C total score >33. Clusters 2 (n=32; anxiety phenotype) and 3 (n=130; mixed anxiety/depression phenotype) reported minimal PTSD symptoms, with modest BAI (Cluster 2) and PHS-9 (Cluster 3) elevations. Cluster 4 (n=39; normative MH phenotype) reported no clinical MH symptom elevations.

Comparisons of explanatory factors between MH phenotypes revealed a modestly higher rate of physical (14.5% vs. 5.1%; P=0.13) and sexual (27.6% vs. 12.8%; P=0.07) abuse among the PTSD phenotype (Cluster 1) vs. the normative MH phenotype (Cluster 4).

**Conclusion:** We identified unique MH phenotypes among PWH and confirmed the importance of ELS, particularly sexual abuse, as an early risk determinant for unfavorable MH among PWH in adulthood. Notably, PWH in the normative MH phenotype also reported a history of sexual abuse, consistent with resilience. Follow-up analyses will have been removed. The figure, table, or graphic for this abstract has been removed.

576 HIV Infection and Alzheimer’s Disease Pathobiology in a Novel Humanized APP-Knock in Mouse Model

Shaurav Bhattachari, Pravin Yeapuri, Jatin Machhi, Yaman Lu, Rana Kadry, Emma G. Foster, Krista L. Hamminga, Emiko M. Waight, Chen Zhang, Prasanta Dash, Santhi Gorantla, Larisa Poluektova, Rodney L. Mosley, Howard E. Gendelman

University of Nebraska Medical Center, Omaha, NE, USA

**Background:** The prevalence of aged-associated Alzheimer’s-like disease is increasing in people living with HIV. Disease mechanisms are linked to interactions between progressive HIV infection, neuroinflammation, CD4+ T cell depletion, and aggregation of misfolded proteins. Sustained viral replication in the brain and immune dysfunction accelerates neuroinflammation. Simultaneous HIV and Alzheimer’s disease (AD) studies have been limited due to the lack of appropriate animal models. A relevant animal model would require human microglia and a robust adaptive immune system in an immune-deficient background amenable to human cell reconstitution.

**Methods:** We created a novel humanized AD mouse using CRISPR-Cas9 technology to address the need for an appropriate animal model to study HIV and AD simultaneously. This was accomplished by knocking in (KI) human APPKM670,671NL, PS1M146V, or MAIPTP3D15 in an immunodeficient NOD mouse (Fig 1A). The APP-KI mice were crossed with NOD/hL34 (NOD/Tg(CMV-IL34)), supporting the development of human microglia. This allows to reconstitute the human innate and adaptive immune system in the brain and periphery (APP/NOD/hL34 mice). APP-KI/NOD/hL34 mice were reconstituted with human hematopoietic progenitor cells to evaluate progressive HIV-1 infection. Four-month-old, humanized mice were infected with a macrophage-tropic HIV-1ADA at a tissue culture infectious dose (TCID50) of 10e4/mice. Mice were sacrificed at four and eight weeks after viral infection.

**Results:** After four weeks of infection, plasma viral load demonstrated productive HIV-1 infection with an average of plasma HIV-1 RNA levels of 8.96e+03 at 2 weeks and 2.82e+05 at 8 weeks after infection. HIV-1p24 was readily detected in the brain (Fig 1B). After four weeks, the insoluble amyloid burden in HIV-1-infected mice was markedly increased compared to uninfected controls as determined by amyloid-beta42 (Aβ42) ELISA (Fig 1C). Immunofluorescence staining revealed co-localization of Aβ fibrils and human IBA1+ microglia. Similarly, IHC staining of the brain showed more reactive microglia in the HIV-infected mice compared to the control mice.

**Conclusion:** We observed that HIV-1 infection accelerates AD pathology by increasing Aβ accumulation. Our results highlight, for the first time, the utility of this humanized CRISPR AD mouse model for evaluating the interconnections between progressive HIV infection and AD pathology.
Increased mtDNA Level in Neuron-Derived Extracellular Vesicles in African Americans With HIV
Vladimir Berthaud, Waldemar Popik, Tarik Smith, Derek Wilus, Venkateswara R. Amara, Franklin Nouvet
MetroHealth Medical Center, Nashville, TN, USA

**Background:** HIV-associated neurocognitive disorders (HAND) remain common among people with HIV (PWH). Cigarette smoking induces mitochondrial damage and is more prevalent among PWH. Mitochondrial DNA (mtDNA) content reduction often occurs before neuronal degeneration. Our objective was to determine the effect of smoking on mtDNA content in neuron-derived extracellular vesicles (NEVs) isolated from the peripheral blood of African Americans (AAs), according to smoking and HIV status. We hypothesized that smoking exacerbates neuronal mtDNA damage in virally suppressed AA PWH, leading to increased release of mtDNA in NEVs.

**Methods:** Twenty-four AA men, aged 45–64, were recruited from Meharry outpatient clinics: HIV-negative non-smokers (NNS), HIV-negative smokers (NS), HIV-positive non-smokers (PNS), and HIV-positive smokers (PS). HIV-positive participants had plasma RNA ≤20 copies/ml. Blood plasma was obtained by ultracentrifugation. NEVs expressing neuron specific L1CAM antigen were isolated from a total pool of EVs. mtDNA content in NEVs was analyzed by qPCR using primer sets that amplify mtDNA’s unique region (D-loop). The copy number of mtDNA amplicons was quantitated using a standard curve. Welch’s One-way ANOVA was applied to determine differences in mean mtDNA content and multiple comparisons done with Games-Howell test, which considers heteroscedasticity. We used multiple linear regression and tested for interaction to determine the association between HIV and mtDNA content, adjusting for smoking status.

**Results:** NEVs mtDNA content were heteroscedastic (P-Value < 0.001). We found significant pairwise differences among NEVs mtDNA groups: between NNS and PNS (P-Value = 0.022), NS and PNS (P-Value = 0.014), NNS and PS (P-Value = 0.006), and NNS and PNS (P-Value = 0.005). Smoking did not significantly contribute to the linear model. Significance was found in HIV status (P-Value = 0.006) and the interaction term between HIV and smoking status (P-Value = 0.011).

**Conclusion:** We found a significant increase in mtDNA level in NEVs of AA PWH compared to HIV-negative counterparts. Smoking increased mtDNA levels in NEVs from AA PWH compared to non-smokers. mtDNA content in NEVs represents a potential biomarker for mitochondrial dysfunction that may precede neuronal damage in virally suppressed AA PWH.

Cerebrospinal Fluid NFL Decreases After Initiation of ART, but Slower Than Inflammatory Biomarkers
Linn Renborg, Aylin Yilmaz, Staffan Nilsson, Henrik Zetterberg, Kaj Blennow, Magnus Grislien
Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

**Background:** Persistent intrathecal immune activation and signs of neuronal disturbances are present in many HIV-infected individuals despite effective antiretroviral treatment (ART). We have studied the decay characteristics of neurofilament light (NFL) protein, a marker of neuronal injury, in cerebrospinal fluid (CSF) after initiation of ART in a large cohort of HIV-infected individuals.

**Methods:** In this longitudinal study, we assessed the levels of NFL, and a panel of neuroinflammatory biomarkers, including YKL-40, sTREM-2, neopterin and GFAp, in consecutive archived CSF samples from 99 people with HIV (PWH) who had achieved viral suppression. Participants were followed from before treatment initiation and up to at least one year on ART. Comparison of means was performed using t-test and partial correlations were calculated adjusting for age.
579 Tryptophan and Kynurenine Pathway Activation and Cognition in Virally-Suppressed Women With HIV

Eran F. Sherer1, Raha M. Dastgheyb2, Audrey L. French3, Elizabeth Daubert2, Ralph Morack4, Claire B. Cline3, Kevin Bullock1, Deborah Gustafson6, Anjali Sharma6, Andrea Rogando1, Ojin Q0, Helen Heubers1, Heather Hur1, Kathleen Weber

1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2The Johns Hopkins University, Baltimore, MD, USA, 3Stroger Hospital of Cook County, Chicago, IL, USA, 4Alpert Einstein College of Medicine, Bronx, NY, USA, 5University of Michigan, Ann Arbor, MI, USA

Abstract: Dysregulated immune function and cognitive complications persist in people with HIV (PWH) despite viral suppression. Inflammatory cytokines and HIV proteins induce the enzyme IDO (indoleamine 2,3-dioxygenase) to convert tryptophan (T) to kynurenine (K) while producing reactive oxygen species and downstream neurotoxic metabolites, which may lead to cognitive complications. Using the KD ratio as a surrogate marker, we investigated the relationship between IDO activation and cognition in virally-suppressed women with HIV (VS-WWH) and women without HIV (WWOh).

Methods: 99 VS-WWH on stable antiretroviral therapy (median age = 54, 73% Black) and 102 (median age = 52, 75% Black) demographically similar WWOh from Chicago and New York sites of the Women’s Intergency HIV Study (WHIS) completed a neuropsychological test battery assessing motor function, processing speed, attention/working memory, verbal fluency, verbal learning and memory, and executive function. Plasma tryptophan and kynurenine were measured using liquid chromatography-tandem mass spectrometry, and targeted plasma monocyte-derived (sCD163, sCD14, MCP-1/CC2) and general inflammatory markers (TNF-α, IL-6, hsCRP, hsl-hsl: 6) were measured using enzyme-linked immunosorbent assay (ELISA).

Results: VS-WWH had a higher KD ratio (P<0.01) and higher sCD14 levels (P<0.05) compared to WWH. In false discovery rate-corrected multivariable regression analyses, higher KD ratio was related to worse motor function in VS-WWW (r = -0.33, P<0.05). This relationship was independent of inflammatory markers. No relationship between IDO activation and motor function was observed in the WWH.

Conclusion: IDO activation was associated with worse fine motor control in VS-WWH independent of measures of systemic inflammation. Further studies are required to investigate the biological mechanisms linking IDO activation to cognitive complications including poor fine motor function among PWH, despite having well-treated HIV.

580 Impact of Recombination on HIV-1 Evolutionary Dynamics in CSF and Plasma

Li Li1, Leslie St. Bernard1, Daniel Dunn2, Douglas F. Nixon1, Weigang Qiu1, Teresa H. Evering2

1Hunter College, New York, NY, USA, 2Weill Cornell Medicine, New York, NY, USA

Background: HIV-1 is capable of establishing distinct viral populations within CNS, leading to a spectrum of neuronal complications. An improved understanding of HIV-1 evolutionary dynamics across the CNS and plasma is necessary to inform vaccine and cure efforts and longitudinal assessments are lacking.

Methods: We used single genome amplification to generate full-length HIV-1 env (>2,500) clade B variants from the paired CSF and plasma of 13 chronically infected individuals living with HIV from the CHARTER cohort with no neurocognitive impairment (N=6) and asymptomatic or mild neurocognitive disease (N=7). Participants were virologic and either treatment naïve (N=6) or experienced (N=7). Each participant contributed viral samples from two time points separated by 6 to 48 months. We used phylogenetic analysis to determine viral compartmentalization and genetic divergence. Recombination was detected using both RDP and ClonalFrameML packages. dN/dS analysis was conducted in DnaSP software to assess selection force. BEAST software was used to estimate env DNA substitution rate.

Results: We analyzed 1300 confirmed single genome sequences. Compartmentalization between CSF and plasma was observed in 11 out of 13 participants in at least one of two time points. In analyses of combined time points, plasma-derived variants showed higher sequence diversity (0.0302 vs 0.0247 in average nucleotide difference, p=0.016), divergence from the origin (0.0830 vs 0.0639 substitutions/site, p=0.041), recombinant frequency (0.147 vs 0.107/seq, p=0.046), and nucleotide substitution rate (5.3±3 vs 1.07±2 sub/site/year, p=0.017) when compared to those from paired CSF. Pervasive recombination was observed across env in plasma-derived variants. The ratio of recombination to point mutation (ρ/θ) was estimated to be 13.86±0.78. The ratio of nonsynonymous to synonymous substitution rate (dN/dS) was estimated to be 0.667±0.042 and 0.813±0.044 in CSF and plasma subgroups, respectively, indicating stronger positive selection on env in plasma compared to CSF (p=0.001).

Conclusion: These findings revealed distinct intra-participant HIV-1 evolutionary dynamics between the CSF and plasma and suggest stronger immune selection on HIV-1 env in the peripheral blood than in the CSF. They also suggest that on average, recombination plays a larger role in HIV-1 env diversification in the plasma than in the CSF, and is the main driver of viral adaptive evolution in the plasma in this cohort with high rates of compartmentalized virus.
depression in PWH is associated with a higher burden of heteroplasmy (HP) (number of mtDNA loci with ≥2 somatic variants) across the mtDNA genome.

**Methods:** We performed Illumina deep mtDNA sequencing on genomic DNA sampled at 2 visits 10 years apart in 92 PWH and 37 PWPH from San Diego-based observational studies (mean coverage depth 3000X). Participants underwent serial neuromedical, mental health (Beck Depression Inventory-II, BDI-II), and comprehensive cognitive assessments. A BDI-II score and cognitive, apathy, affective and somatic depressive symptom scores were obtained; demographically adjusted cognitive T-scores were converted to domain deficit scores (DDS). Plasma MCP-1 was measured by immunoassay. Raw reads were mapped to revised Cambridge Reference Sequence, and HP was called at the 1-99% threshold using the GATK Mutect2 pipeline. Change in mtDNA HP burden at 10 years was compared by HIV status, adjusting for influential covariates (univariate p<0.05). Associations of HP burden at 10 years with BDI-II scores or depression (BDI-II score ≥14) were tested in backward-elimination Poison regression models, using the Akaike Information Criterion.

**Results:** PWH (mean age 44, 11% female, 60% arievermic, 26% depressed) had higher plasma MCP-1 and more prevalent substance use than PWH (mean age 45, 23% female). PWH did not acquire higher HP burdens over 10 years than PWOH (adjusted beta = 2.75, p = 0.337 for PWH). Higher mtDNA HP at 10 years was associated with more depression (adjusted beta 0.320, p < 0.001), and higher BDI-II score (beta 0.014, p < 0.001), adjusting for age, sex, race, mean coverage, HIV status, hemoglobin, smoking, plasma MCP-1, substance use, tenofovir alafenamide use, eglobal cognitive impairment (DDS<0.5), with similar results in PWH. In PWH, higher HP burden was also associated with higher BDI-II-based cognitive, apathy, affective, and somatic depressive symptom scores (adjusted beta 0.024-0.056; all p-values ≤0.01).

**Conclusion:** PWH do not accumulate HP across the mtDNA genome faster than PWOH over a decade, but higher overall mtDNA HP burden may confer increased risk for depressive symptoms and depression, regardless of HIV status. Future studies should define the mechanisms for these associations.

### 583 Sociodemographics Were Stronger Classifiers of Cognitive Profiles Than Neuroimaging in HIV

Raha M. Dastgheyb, Sarah Cooley, Alison Buchholz, Kalem J. Petersen, Beau Ances, Leah H. Rubin
1The Johns Hopkins Hospital, Baltimore, MD, USA, 2Washington University in St Louis, St Louis, MO, USA, 3The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4The Johns Hopkins University, Baltimore, MD, USA

**Background:** Despite advancements in antiretroviral therapy, cognitive issues persist in some people with HIV (PWH) and are heterogeneous in nature. Recent research has focused on understanding cognitive profiles present in PWH and identifying factors that distinguish between these profiles; however, neuroimaging metrics of brain structure and function are rarely incorporated into these analyses.

**Methods:** We identified cognitive profiles among 225 PWH (mean age=51; 21% female, 67% African American/Black; mean years of education=13.3) from a single site (Washington University in St. Louis). Participants completed a standard neuropsychological test (NP) battery of 10 tests (16 outcomes) and magnetic resonance imaging (MRI), including structural MRI to measure regional volumes and resting-state functional MRI to assess brain connectivity. Raw NP test scores were converted to scaled scores and then Kohonen self-organizing maps were used to classify individuals with similar cognitive profiles. Socio-demographic features (including age, race, education, premorbid IQ (WRAT-3), and area deprivation index), HIV clinical factors (nadir and recent CD4 count, recent CD4 count, CD4/CD8 ratio, viral load), hepatitis C co-infection, CD4 count, recent CD8 count, CD4/CD8 ratio, viral load), hepatitis C co-infection, CD4 and CD8 count, nadir CD4, HIV duration), a neuropsychological assessment, a structural brain MRI scan, and a 20-minute single-voxel MRS in the frontal white matter, right caudate, and posterior cingulate with whole brain T1 and T2 mapping to derive the absolute concentration of major brain metabolites. MRS data were fitted with the spant R package and scaled using partial volumes (derived from Freesurfer and FSL MRS tools structural volumes). Mixed effect models assessed associations and longitudinal changes to T1, T2, and metabolite concentrations with age, baseline HIV markers, and HIV-associated cognitive disorder (HAND).

**Results:** Higher T2 but not T1 values, increased with age in white and grey matter (p < 0.002). HAND was associated with higher T2 values over time (p < 0.03). Older adults with HAND had higher T2 values than older adults without HAND (p < 0.04). Myo-Inositol concentration increased in all brain regions as a function of increased age (p < 0.01). There was a significant age-by-nadir CD4 interaction in frontal white matter for myo-Inositol (p < 0.001). Lower baseline CD4+ cell count and HAND were associated with lower glutamate in the cingulate (p = 0.04) and caudate (p = 0.003) of older participants, respectively.

**Conclusion:** Our results confirm and expand the evidence for chronic and slowly progressive structural and neurometabolic abnormalities including age-related loss of brain tissue density, age and HAND-related inflammation/glial activation, legacy-related (nadir CD4) inflammation/glial activation, but also age, current immune function (baseline CD4) and HAND related excitoexcitotoxicity in the cortex and basal ganglia. Our 20-minute absolute concentration MRS scan can be clinically scalable to monitor HAND stability or progression in virally suppressed PWH who are aging.

### 584 MRI With T2 Maps and Spectroscopy Show Chronic Progressive Brain Damage Despite HIV Suppression

David Jakabek, Kurt Lancaster, Lauriane Juge, Caroline D. Rae, Lucette A. Eyquem, Bruce J. Brew
1St Vincent’s Hospital, Sydney, Australia. 2University of New South Wales, Darlington, Australia

**Background:** We used a 20-minute absolute single-voxel proton magnetic resonance spectroscopy (MRS) scan with relaxometry (T1 and T2 maps) to better quantify water as a reference for major brain metabolites in virally suppressed people living with HIV (PWH) who are aging and were assessed for HIV immune markers and cognitive functions.

**Methods:** 39 PWH, all male (mean age: 53 ± 14, 60 + years old; 35%, HIV duration: 18 years, 20% nadir CD4 <200 cp/mL) were enrolled into a prospective study investigating brain metabolites during long-term viral suppression. They completed a baseline and a two-year follow-up clinical visit for HIV biomarkers (CD4 and CD8 count, nadir CD4, HIV duration), a neuropsychological assessment, a structural brain MRI scan, and a 20-minute single-voxel MRS in the frontal white matter, right caudate, and posterior cingulate with whole brain T1 and T2 mapping to derive the absolute concentration of major brain metabolites. MRS data were fitted with the spant R package and scaled using partial volumes (derived from Freesurfer and FSL MRS tools structural volumes). Mixed effect models assessed associations and longitudinal changes to T1, T2, and metabolite concentrations with age, baseline HIV markers, and HIV-associated cognitive disorder (HAND).

**Results:** Higher T2, but not T1 values, increased with age in white and grey matter (p < 0.002). HAND was associated with higher T2 values over time (p < 0.03). Older adults with HAND had higher T2 values than older adults without HAND (p < 0.04). Myo-Inositol concentration increased in all brain regions as a function of increased age (p < 0.01). There was a significant age-by-nadir CD4 interaction in frontal white matter for myo-Inositol (p < 0.001). Lower baseline CD4+ cell count and HAND were associated with lower glutamate in the cingulate (p = 0.04) and caudate (p = 0.003) of older participants, respectively.

**Conclusion:** Our results confirm and expand the evidence for chronic and slowly progressive structural and neurometabolic abnormalities including age-related loss of brain tissue density, age and HAND-related inflammation/glial activation, legacy-related (nadir CD4) inflammation/glial activation, but also age, current immune function (baseline CD4) and HAND related excitoexcitotoxicity in the cortex and basal ganglia. Our 20-minute absolute concentration MRS scan can be clinically scalable to monitor HAND stability or progression in virally suppressed PWH who are aging.

### 585 Functional Brain Network Changes Among People With HIV and Viral Suppression

Jacob Van Doorn, Leah H. Rubin, Farah Nasaz, Liuyi Chen, Seble Kassa, Lakshmi Goparaju, Raha M. Dastgheyb, Asante Kamkwala, Hannah Lee, Arianna Konstantopoulou, Joan Severson, Olusola Aijilore, Alex Lee, Pauline Mack, University of Illinois at Chicago, IL, USA. 2The Johns Hopkins University, Baltimore, MD, USA, 3University of New South Wales, Darlinghurst, Australia

**Background:** The functional connectivity of global brain networks, quantified using graph theory metrics, provide insights into the efficiency and other characteristics of brain network functioning at a whole brain level. Such metrics are widely studied in numerous neurological and psychiatric conditions under a variety of task demands but have not yet been evaluated in virally-suppressed people with HIV (VS-PWH). Here we examined global brain network...
organization in virally-suppressed people with HIV under three functional MRI conditions including resting state MRI (rsfMRI) and the performance of verbal memory and go/no-go tasks.

**Methods:** A total of 56 participants (69.6% VS-PWH; 51.7% female, 69.6% black) completed the MRI session and performed a battery of cognitive tests using the iPad-based platform Brain-Baseline Assessment of Cognition and Everyday Functioning. Graph theory metrics of the global functional brain network were computed in the weighted positive network, determined by positive correlations in the BOLD activity, to yield assortativity, characteristic path length (CPL), clustering coefficient (CC), global efficiency (GE), modularity (Q), and average node strength (ANS). Multiple regressions were performed to determine serumstatus and associations with cognitive performance, adjusting for sex, age, race, ethnicity and education.

**Results:** In resting state, compared to controls, VS-PWH showed lower CC (p<.03), GE (p=.02), and ANS (p=.02) and higher CPL (p=.04) and Q (p=.04). In the verbal memory task, compared to controls, VS-PWH also showed lower CC (p=.01), GE (p=.02), and ANS (p=.01) and higher CPL (p=.04) and Q (p=.005). No HIV-serostatus effects were observed in the go/no-go task. In the total sample, there were significant associations of CPL, CC, GE, and ANS with processing speed (reaction time on flanker, Stroop, Trail Making, and fine motor skills task), working memory (accuracy on n-back) and visual spatial learning test (total correct across trials).

**Conclusion:** During both the resting state and performance of a verbal memory task, VS-PWH show less efficiently organized network structure (GE), less small-world network structure (CC, CPL), and less distributed connectivity (Q and ANS). These metrics relate to a number of cognitive processes including processing speed, working memory, and visual spatial learning. Here we show graph theory metrics may serve as biomarker for VS-PWH to monitor disease progress and to evaluate new therapeutics.

586 HIV Status and APOE4 Differentially Impact White Matter Integrity in Adults With HIV

**Peyton Thomas**1, Hannah Walsh1, Richard C. Gallagher1, Kyle Shattuck1, Princy Kumar1, David J. Moore2, Ronald J. Ellis2, Ronald J. Ellis2, Xiong Jiang3

1Georgetown University Medical Center, Washington, DC, USA, 2University of California San Diego, La Jolla, CA, USA

**Background:** The median age of people with HIV (PWH) in USA is now over 50. This aging HIV+ population is facing increasingly significant risk of other neurodegenerative diseases-in addition to HIV brain disease-especially Alzheimer’s disease. We investigated the potential impact of HIV-disease, APOE4 (E4), and their interactions on white matter (WM) microstructure in a cohort of middle-aged to older PWH.

**Methods:** Seventy-six adults (44-69 y.o. (56±6±3), 24 female, 58 PWH) participated in this study. All diffusion weighted images were preprocessed in FSL then analyzed using the automated fiber quantification (AFQ) software in Python. AFQ produced fractional anisotropy (FA) and mean diffusivity (MD) values at each of 100 equidistant nodes along each of 22 major WM bundles. We analyzed group differences along each tract for both FA and MD using pointwise t-tests with FDR correction to investigate the impact of HIV and E4 status on FA and MD values. Significant regions of group differences were defined by pFDR<0.05 with at least two adjacent nodes. Region-of-interest (ROI) based analyses were conducted on these significant regions to investigate the interactions between age, E4, and HIV-disease.

**Results:** Compared to controls, PWH showed trends of increased MD along all 22 bundles, with significant regions of group differences along the left inferior frontal occipital (Fig. A) and left uncinate tracts. ROI-based analyses revealed significant interactions between HIV-status and age in all these regions (at least p<.004), with a steeper age-related increase in MD in PWH. Across PWH and controls, E4 carriers had significantly reduced FA in multiple regions of the left arcuate fasciculus versus non-carriers (Fig. B). In PWH, the E4 effect remained significant (p<.001), and importantly, ROI-based analyses revealed significant interactions between E4-status and HIV disease duration in most of these regions, with a steeper disease duration-related decline in FA in PWH E4 carriers (after controlling for age, p<.045).

**Conclusion:** HIV and APOE4 differentially impact white matter microstructure in middle-aged to older adults with HIV. Specifically, HIV-disease is associated with wide-spread increases in MD, and older PWH are disproportionally affected. By contrast, E4-associated FA reduction is more localized, and PWH E4 carriers with long disease duration are at a greater risk. These findings warrant further examination of more localized WM changes along individual tracts in the rapidly aging HIV+ population.

A Left Inferior Frontal Occipital

B Left Arcuate Fasciculus

**Figure:** Tract profiles (top) with significant group differences (p<0.05, FDR corrected) in gray. Lines represent group mean values (MD on left, FA on right, ± SEM). Corresponding anatomical locations exemplified in bottom.

587 HIV Clinical Factors and Cardiovascular Disease Risk Predict White Matter Hyperintensity Burden

**Mikaley Bolden1, Peyton Thomas1, Richard C. Gallagher1, Princy Kumar1, David J. Moore2, Ronald J. Ellis1, Xiong Jiang2

1Georgetown University Medical Center, Washington, DC, USA, 2University of California San Diego, La Jolla, CA, USA

**Background:** Cardiovascular disease (CVD) risk factors such as hypertension are highly prevalent in people with HIV (PWH) and have been shown to affect brain function and structure, including an elevated risk of white matter hyperintensity (WMH), a measure of small vessel disease. We investigated the factors associated with WMH burden (measured as total WMH volume, or WMHv) in adults with chronic HIV disease, focusing on CVD risk and HIV-clinical factors.

**Methods:** Participants were 134 adults (age range 40-70, 34 females, 82 African American, 97 PWH). CVD percent risk was calculated using the Framingham General Cardiovascular Risk Profile, including age, BMI, SBP status and treatment, smoking status, and diabetes status. High-resolution T1w structural MRI images (3x1x1mm3) were acquired. The software package CAT12 (https://neuro-jena.github.io/cat/) was used to detect WMHs and obtain WMHv and gray matter volume (GMv). The effects of HIV status, age, sex, race, education, and CVD percent risk on WMH burden were examined using non-parametric ANCOVA. For PWH, potential impacts of CD4 nadir, current CD4/CD8 ratio, and disease duration on total WMHv were examined using non-parametric regressions separately for each clinical factor. Parametric multiple linear regressions investigated the effects of WMHv, HIV status, age, sex, race, education, and HIV clinical factors on total GMv. Total WMHv and GMv were normalized by intracranial volume.

**Results:** In the full study sample, higher CVD percent risk, older age, and African American race strongly predicted higher WMHv (at least p=.001), versus a marginal effect of education (p=.049) and HIV status (p=.057), while higher WMHv, male sex, and older age were associated with lower GMv (at least p=.001). In PWH, in addition to CVD percent risk, age, and race, lower CD4/CD8 ratio (p=.016) and longer HIV disease duration (p=.002) also strongly predicted higher WMHv.

**Conclusion:** Consistent with previous findings, WMHv is prevalent in middle-aged to older PWH on cART and with successful viral suppression, and WMH burden strongly correlates with CVD risk and age. In addition, WMH burden correlates with current CD4/CD8 ratio and HIV disease duration, suggesting the need to improve immune function, control chronic neuroinflammation, and develop antiretroviral regimens that are less toxic to the CNS. Also, the significantly higher WMH burden in African Americans with and without HIV calls for actions to address health disparities.
Neurite Orientation Dispersion and Density Imaging in People With HIV on Stable Treatment

Phillip Chan, Nakul Raval, Praveen Honhar, Jennifer Chiarella, Allison Nelson, David Matuskey, Richard Carson, Ansel Hillmer, Serena Spudich

Yale University, New Haven, CT, USA

Background: Neurite Orientation Dispersion and Density Imaging (NODDI) is a multi-parametrical diffusion MRI technique that characterizes brain microstructures by quantifying the packing density of axons or dendrites (neurite density index, NDI); the orientational coherence of neurites (orientation dispersion index, ODI) and the free water fraction (isotropic compartment, ISO). This preliminary study compares NODDI metrics cross-sectionally between virally suppressed people with HIV (PWH, plasma HIV RNA <20 cps/ml, median duration of treatment: 22 years) and people without HIV (PW0H), and longitudinally in the PWH group over a median duration of 32 months.

Methods: Eight PWH (age: 56 [IQR 53,59], 8/8 male) and eight PW0H (age: 57 [IQR 53,62], 3/8 male) underwent NODDI scans in a 3T Siemens Prisma scanner, employing a two-shell diffusion protocol tailored for NODDI (32,64 direction; b=700,2000 s/mm²; 80 slices; TR/TE=4100/88ms). After performing distortion and eddy-current correction and FreeSurfer segmentations, NDI, ODI and ISO were measured in the primary region of interest (ROI), the frontostriatothalamic (FST) region, while NDI was measured in the secondary ROIs, including the frontal, parietal, temporal, and occipital lobes. Statistical assessments included the Mann-Whitney U Test for cross-sectional evaluations and the Wilcoxon Signed Rank Test for longitudinal changes.

Results: At the 1st scan, the CD4+ and CD8+ T-cell counts of PWH were 631 [IQR 412,831] and 576 [IQR 442,840] cells/mm³, with a CD4/CD8 ratio of 0.96 [IQR 0.66,1.36]. Compared to PW0H, PWH had lower NDI (mean difference Δ=−13.6±6.6%, p=0.001) and ODI (Δ=−12.1±3.7%, p=0.001), but ISO showed no significant difference in the FST region. PWH also had lower NDI in all secondary ROIs when compared to PW0H (Δ<0.05). In the longitudinal analysis, PWH showed a decrease in NDI (Δ=−11.2±9.2%, p=0.02) and ISO (Δ=−12.1±7.6%, p=0.007) but statistically unchanged ODI (Δ=1.5±7.8%, p=0.547) in the FST region. Moreover, NDI decreased in all secondary ROIs (Δ<0.05).

Conclusion: Compared to PW0H, PWH with stable HIV suppression had lower NDI and ODI in the FST and lower NDI in major cortical regions, indicating a possible loss in microstructural integrity. The longitudinal decline of NDI in PWH may indicate changes occurring at the axonal/dendritic level. Future inclusion of cognitive testing, multi-modal neuroimaging, and longitudinal PWH controls will help validate and understand the observed NODDI changes.

Associations Between a Polynomic Risk Score for Alzheimer’s Disease and Brain Integrity in HIV

Anna Curtis, Laura Ibanez, Carlos Cruchaga, Beau Ances, Sarah Cooley

Washington University at St Louis, St Louis, MO, USA

Background: People living with HIV (PWH) continue to live to older ages making it difficult to differentiate between age-related neuropsychiatric disorders, such as Alzheimer disease (AD), and HIV-related neuropsychiatric impairment. Polynomic risk scores (PRS) allow for computing genetic risk for various diseases. This study leveraged a PRS calculated using the latest genome-wide association study for AD (AD-PRS) to evaluate if it was associated with the presence of HIV disease markers, HIV-related neurocognitive impairment, and brain integrity.

Methods: 115 PWH (age=50, male n=94, female n=21) completed magnetic resonance imaging, a blood draw, and a comprehensive neuropsychological battery comprised of 15 tests covering 5 cognitive domains (learning, recall, executive functioning, psychomotor speed, and language). Participants were genotyped with an Illumina array platform (GSA), stringent quality control and imputation was performed following standard pipelines. AD-PRS was computed using the binary logistic transformation of the reported OR from the sentinel SNPs from the latest AD GWAS, including the APOE region, using PRSice. Regression analysis (adjusted for age, sex, and race) was used to examine relationships between PRS and cognitive test and domain Z-scores, inflammatory markers (sCD14, sCD163), brain volumes, cerebral blood flow (CBF), and HIV variables (CD4+ cell count, CD8 t-cell count, CD4:CD8 ratio, duration of infection).

Results: Higher AD-PRS values were associated with worse cognitive test scores for letter number sequencing (p=0.015), animal fluency (p=0.039), verb fluency (p=0.001) and language domain (p=0.024). There was a negative correlation between AD-PRS and inflammatory markers sCD163 (p=0.043) and sCD14 (p=0.03), and AD-PRS and total gray volume (p=0.032). A significant positive association between the AD-PRS and CBF in the caudate (p=0.047) and putamen volume (p=0.017) was found. No association was found between AD-PRS and HIV variables.

Conclusion: While no change in brain integrity was observed within cognitive domains or brain regions typically affected by AD, an elevated genetic risk measured by AD-PRS did associate with some worse cognitive performance. This mirrors patterns in the general population, implying that certain cognitive deficits among older PWH may result from aging rather than HIV-related factors. AD-PRS also had no association with HIV clinical markers, supporting previous work suggesting that HIV and AD affect brain integrity via different processes.

Further Evidence of Hypertrophy in Acute Infection Identified Using Machine Learning

Jacob Bolzenius, Napapon Sailsutla, Phillip Chan, Carlo P. Sasaki, Julie Ake, Somchal Sriplienchan, Lydie Trautmann, Sandhya Vasan, Trevor A. Crowley, Ferron F. Ocampo, Serena S. Spudich, Victor Valcour, Kilian Poft, Robert Paul

For the SEARCH1001/RV254 Study Team

University of Missouri St Louis, St Louis, MO, USA, University of Hawaii, Honolulu, HI, USA, Yale University, New Haven, CT, USA, S i m i l a r i s c h e n , Bangkok, Thailand, Walter Reed Army Institute of Research, Silver Spring, MO, USA, US Military HIV Research Programs, Silver Spring, MO, USA, Henry M Jackson Foundation, Bethesda, MD, USA, University of California San Francisco, San Francisco, CA, USA, Stanford University, Stanford, CA, USA

Background: A recent study of structural brain volumes during acute HIV infection (AHI) revealed evidence of hypertrophy among individuals in Fiebig stages III-V compared to Fiebig I-II. The present study incorporated multiple neuroimaging modalities (structure and function), blood markers of inflammation and immune dysregulation, and psychosocial determinants of health using ensemble machine learning to more comprehensively characterize brain integrity in late vs. early AHI and discover potential explanatory factors.

Methods: 112 Thai males with AHI (age 20-46) enrolled in RV254/SEARCH010 were included in the analysis. Features classifying individuals into early (Fiebig I-II; n=32) vs. late (Fiebig III-V; n=80) AHI were ranked using gradient-boosted multivariate regression (GBM) with repeated cross validation. Predictors included demographic, HIV disease, cognitive, neurologic exam, mental health, substance use, plasma immune/inflammatory, and multimodal 3T neuroimaging (i.e., volumes, resting state connectivity, diffusion, and spectroscopy) indices collected at enrollment.

Results: The GBM identified a combination of variables that classified individuals into early vs. late Fiebig with an average F1 score (a metric of model performance) of .82. Individuals in late Fiebig stages exhibited larger regional volumes in the olfactory cortex, putamen, raphe nucleus, and pallidum. Smaller performance) of .82. Individuals in late stages exhibited larger regional volumes in the olfactory cortex, putamen, raphe nucleus, and pallidum. Smaller volumes among late Fiebig individuals were noted in the ventral temporal area, along with lower blood neutrophil percent and high-density lipoproteins, greater functional connectivity between the right executive control network and visuospatial networks, and lower use of erectile dysfunction medication.

Conclusion: Findings demonstrate that brain alterations in late vs. early stages of AHI associate with distinct multidimensional parameters: larger regional volumes observed together with lower immune levels (i.e., neutrophils) suggest that viral-immune dysregulation already underway in late Fiebig stages manifests as hypertrophy via neuroinflammation. Additionally, we reveal evidence of brain-behavior relationships via recent substance use and ventral tegmental area volume, a region implicated in addictive behaviors. Resting state connectivity differences may also reflect disrupted structural/functional integration. Psychosocial and immune processes operative during the first weeks of AHI and their effects on brain integrity may explain variability in long-term outcomes for those starting ART at later stages.
591 Beta-Amyloid PET Positivity Among Cognitively-Impaired People With HIV Over Age 60

Samuel Wilson1, Andjelika Milicic1, Shireen Javandel2, Claire Yballa2, Benedetta Milanin1, Kilian Pohl2, Robert Paul2, Victor Valcour2, Andjelika Milicic2, Alina Pang3, Shireen Javandel2
1Thomas Jefferson University, Philadelphia, PA, USA, 2University of California San Francisco, San Francisco, CA, USA, 3Inovigate GmbH, Basel, Switzerland, 4Stanford University, Stanford, CA, USA,
5University of Missouri St Louis, St Louis, MO, USA

Background: Amyloid positron emission tomography (PET) imaging can stratify risk for Alzheimer’s disease (AD). Limited data exist among people with HIV (PWH) and HIV-related neurocognitive disorder (HAND) in older age groups. In this analysis, we sought to characterize frequency of amyloid PET positivity among older cognitively-impaired PWH compared to cognitively healthy people without HIV (controls).

Methods: Virally suppressed PWH were enrolled in a study of HAND where PET positivity was used to exclude the possibility of AD (exclusion criteria). Participants underwent a standardized neurophysiological battery to diagnose HAND. Healthy controls were identified and matched by age from a separate cohort at our site. No participant from either group showed clinical signs and symptoms in a pattern that would be concerning for AD. We completed amyloid PET imaging using florbetapir F-18 among both groups which were visually read as amyloid positive (PET+) or negative (PET-).

Results: Compared to controls (n=65), the PWH group (n=74) was predominantly male (94.6% vs 72.3%, p<0.001), of non-Hispanic white ethnicity (74.3% vs 83.1%, p=0.211) and reported lower educational attainment (16.2 vs. 17.4 years, p<0.001). Among them, 6 (8.1%) had PET+ scans compared to 14 of 65 matched healthy controls (21.5%, p=0.024). Within the PWH group, we did not identify differences in the neuropsychological testing pattern by PET status (all p-values >0.05).

Conclusion: Relative to healthy controls, this group of cognitively impaired PWH did not show increased frequency of amyloid PET+ compared to controls nor differences in patterns of cognitive performance.

592 Differential Expression Analysis Reveals Pervasive Transcriptional Changes in the Brains of PWH

Kriti Agrawal1, Jay S. Stanley1, Junchen Yang1, Nicholas C. Jacobs1, Haowei Wang1, Le Zhang1, Mark Gerstein2, Yuval Kluger2, Serena Spudich1, Yale University, New Haven, CT, USA

Background: Central nervous system (CNS) dysfunction is a common consequence in people with HIV (PWH). Understanding of the impact of HIV on the brain remains limited to select cell types and regions. We describe preliminary efforts to establish cell-type specific gene expression patterns across multiple regions in PWH using single cell transcriptomics.

Methods: Nuclei were isolated from frozen post-mortem tissue from insular cortex (INS), frontal cortex (PFC), and ventral striatum (VST) from 6 PWH and 6 HIV-negative control (CTR) donors. We profiled transcriptomes and chromatin accessibility profiles of these 36 samples using 10x Genomics Chromium Single Cell Multimodal ATAC+Gene Expression. In total, we analyzed single-cell transcriptomes of 144,918 cells from PWH and 153,868 cells from CTR. An integrated single cell t-SNE embedding was created for each region, and cells were clustered using Louvain. These clusters were then manually annotated to identify major cell types. We also performed pseudobulk differential expression (DE) analysis between PWH and CTR within major cell types and regions using EdgeR.

Results: PWH and CTR donors were similar in age (median 45 and 39 years) and gender (33% vs 17% female) (p>0.1). Canonical neuronal and non-neuronal cell types were detected in all regions. However, a population of putative T-like cells expressing CD247 and SKAP1 was identified in INS of 6 CTR and 5 HIV donors, with >80% of cells annotated as T-like originating from PWH. Consistent with their role as resident immune cells of the CNS, microgliola exhibited the largest gene expression changes in PWH, including upregulation of pathways related to cytokine signaling and innate immunity. On the other hand, many genes were significant on DE analysis (p ≤ 0.05) across all regions (151 PWH, 55 CTR) and multiple cell types (567 PWH, 974 CTR). For example, SERPIN A3 was differentially expressed in PWH in INS, VST, and PFC by astrocytes, endothelial cells, neuronal cells, oligodendrocyte precursor cells, oligodendrocytes, microglia, and pericytes. Despite these widespread trends, 562 of the top 1000 significant results were genes differentially expressed in INS.

Conclusion: We identified patterns of differential cell type abundance and gene/pathway expression in PWH. While our findings are consistent with previous single cell observations of glial cell involvement in PWH, our results indicate that many other cell types are affected by HIV and implicates the INS as a significant region for future exploration.

593 Epigenetic Impact in CSF Cells From 48 Weeks of Adjunctive Telmisartan in Acute HIV

Michael L. Corley1, Phillip Char1, Eugene Kroon2, Napapon Sailaju1, Alina Pang3, Nittaya Phanuphak4, Jennifer Chiarella1, Sandhya Vasan5, Robert Paul2, Lydie Trautmann2, Serena S. Spudich1, Lishomwa Ndhlovu6, for the SEARCH018/RV408 Study Group

1Weill Cornell Medicine, New York, NY, USA, 2Yale University, New Haven, CT, USA, 3SEARCH, Bangkok, Thailand, 4University of Hawaii at Manoa, Honolulu, HI, USA, 5Henry M Jackson Foundation, Bethesda, MD, USA, 6University of Missouri St Louis, St Louis, MO, USA

Background: Telmisartan, an angiotensin II receptor antagonist, is known to reduce inflammation. In a randomized trial, we examined whether 48 weeks of sustained adjunctive telmisartan initiated with antiretroviral therapy (ART) in acute HIV infection (AHI) would modify HIV’s impact on the central nervous system (CNS). While we observed no significant changes in soluble markers of CNS inflammation or injury markers due to telmisartan, we did not evaluate its effects on CNS cells. Hence, we investigated whether telmisartan modified epigenetic states in cerebrospinal fluid (CSF) cells.

Methods: We utilized a new ultra-low DNA input assay to measure genome-wide DNA methylation (DNAm) epigenetic profiles in CSF cells at 48 and 72 weeks from 21 participants with AHI that were randomized 2:1 to initiate treatment with ART+ /- telmisartan for 48 weeks. We used R statistical software to analyze DNA methylation data and identify differentially methylated loci using a linear model with FDR correction.

Results: The median age of participants was 29 years (IQR: 24-34), pre-ART median log2 plasma viral load was 5.95 copies/mL (5.36-6.48), pre-ART median CSF HIV RNA was 2.82 copies/mL (2.17-4.36), and pre-ART CD4+ T cell count was 479 cells/mm³ (95-688). Age, Fiebig stage, pre-ART plasma and CSF viral loads, and pre-ART CD4+ T cell count did not significantly differ between participants randomly assigned to ART+ /- telmisartan versus ART only groups. Comparing the DNAm states of CSF cells at Week 48 in participants receiving ART+ /- telmisartan versus ART only, we identified 11,433 differentially methylated loci using a linear model with FDR correction.

Conclusion: We identified patterns of differential cell type abundance and gene/pathway expression in PWH. While our findings are consistent with previous single cell observations of glial cell involvement in PWH, our results indicate that many other cell types are affected by HIV and implicates the INS as a significant region for future exploration.

largest gene expression changes in PWH, including upregulation of pathways related to cytokine signaling and innate immunity. On the other hand, many genes were significant on DE analysis (p ≤ 0.05) across all regions (151 PWH, 55 CTR) and multiple cell types (567 PWH, 974 CTR). For example, SERPIN A3 was differentially expressed in PWH in INS, VST, and PFC by astrocytes, endothelial cells, neuronal cells, oligodendrocyte precursor cells, oligodendrocytes, microglia, and pericytes. Despite these widespread trends, 562 of the top 1000 significant results were genes differentially expressed in INS.

Conclusion: We identified patterns of differential cell type abundance and gene/pathway expression in PWH. While our findings are consistent with previous single cell observations of glial cell involvement in PWH, our results indicate that many other cell types are affected by HIV and implicates the INS as a significant region for future exploration.
found that only 5.46% of DML identified at week 72 overlapped with changes observed at week 48.

**Conclusion:** Telmisartan initiated during AHI affects epigenetic states of genes related to inflammation, immune response, and cellular proliferation in CSF cells. Telmisartan’s effects on long-term epigenetic reprogramming of CNS cells and CNS outcomes warrant further investigation.

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**594 Monocyte Epigenetic Age Relates to Non-Somatic Depressive Symptoms in Women With Undetectable HIV**

Kalen J. Petersen, Nicole Perez, Ke Xu, Yannan Xu, Lang Lang, Kathryn Anasters, Maria L. Alcайдe, Mandge H. Cohen, Sadeep Shrestha, Andrew Edmonds, Jacqueline Meyers, Seble Kassaye, Beau Ancel, Brad Aus泽ner, Leah H. Rubin

1. Washington University in St Louis, St Louis, MO, USA, 2. New York University, New York, NY, USA

**Background:** HIV and its neuropsychiatric complications have been linked with accelerated biological aging using several DNA methylation-based epigenetic clocks. Monocytes, which are essential to the innate immune response, may contribute to this biological aging effect. A recently developed epigenetic clock derived from monocytes showed increased aging in people with HIV who were heavy alcohol users. However, its relationship with depression is unknown. As depression disproportionately affects women, we examine epigenetic clocks as biomarkers of depressive symptomatology in women with HIV (WWH) and women without HIV (WWoH). Specifically, we focus on the link between epigenetic age and dimensions of depression (non-somatic [affective vs. somatic] vs. cognitive) as the phenotypic expression of depression is heterogeneous.

**Methods:** DNA methylation data and Center for Epidemiological Studies Depression Scale (CES-D) scores were available from 440 Women’s Interagency HIV Study participants. Illumina MethylationEPIC microarrays were used to measure methylation levels in bisulfite-converted DNA from whole blood. Two measures of biological age were calculated (Horvath age and monocyte age). Monocyte DNA methylation (DNAm) patterns indicate a trend towards somatic aging in WWH compared to WWoH aged 50 and older in clinical care for HIV management at Weill Cornell in NYC, were recruited to participate in a detailed biopsychosocial survey, and those age 55 and older were invited to complete an in-person study visit which included blood draw, Montreal Cognitive Assessment (MoCA) and Fried frailty phenotype testing. Genome-wide DNAm was measured from dried blood spots using the Illumina MethylationEPIC platform and analyzed using PhenoAge epigenetic age.

**Results:** A total of 164 participants enrolled in the study; 158 had available biospecimens for DNAm analysis. Median age was 60 years (IQR 56-64), 52 (33%) identified as female, and 76 (50%) identified as Black, median CD4+ T-cell count was 588 cells/mm³ (IQR 323-811) and 93% were suppressed on ART. Epigenetic age analysis indicated an average epigenetic “age advancement” (EAA) of 5.4 years (SD 6.6). Median MoCA score was 24 (21-27) and 67% were pre-frail or frail. There were 17 deaths (10.7% mortality rate) from September 2016-September 2023. EAA was inversely related to chronological age (β= -0.31 [95% CI: -0.48, -0.14] (p<0.01) and was associated with lower MoCA T-cell count (β= -0.84 [95% CI: -1.14, -0.53] for every 100 CD4+ T-cells (p<0.01)) in an unadjusted linear regression model. EAA was associated with lower MoCA score in a linear regression model adjusted for age, sex and race (p<0.01), and there was a trend towards EAA association with more advanced frailty state in an ordinal logistic regression model adjusted for age, sex and race (p=0.07). Survival differed by EAA, and greater advancement was associated with 7-year mortality in a Cox Proportional Hazard model adjusted for age (HR 1.10 [95%CI: 1.02, 1.18] p=0.01) (Figure 1).

**Conclusion:** In OPWH the average EAA was 5.4 years, as calculated by PhenoAge, and was associated with cognitive dysfunction, more advanced frailty state, as well as 7-year mortality. These results suggest epigenetic clocks are a valuable biomarker of aging-related pathologies in OPWH and warrant further study.

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**595 Epigenetic Age Advancement Is Associated With Cognition, Frailty, and Mortality in Older PWH**

Carrie Johnston, Alina P. Pang, Eugenia Siegler, Chelsie Burchett, Charlene Thomas, Mia Crowley, Rochelle O’Brien, Lishomwa Ndhlovu, Marshall Glesby, Michael J. Corley

Weill Cornell Medicine, New York, NY, USA

**Background:** DNA methylation (DNAm) patterns indicate a trend towards epigenetic age advancement in older persons with HIV (OPWH), however, data on relationships with phenotypic changes are limited. We investigated associations between phenotypic measures of cognition, frailty, and 7-year survival with epigenetic age estimates derived from the modification of DNAm in a population of OPWH.

**Methods:** OPWH aged 50 and older in clinical care for HIV management at Weill Cornell in NYC, were recruited to participate in a detailed biopsychosocial survey, and those age 55 and older were invited to complete an in-person study visit which included blood draw, Montreal Cognitive Assessment (MoCA) and Fried frailty phenotype testing. Genome-wide DNAm was measured from dried blood spots using the Illumina MethylationEPIC platform and analyzed using PhenoAge epigenetic age.

**Results:** A total of 164 participants enrolled in the study; 158 had available biospecimens for DNAm analysis. Median age was 60 years (IQR 56-64), 52 (33%) identified as female, and 76 (50%) identified as Black, median CD4+ T-cell count was 588 cells/mm³ (IQR 323-811) and 93% were suppressed on ART. Epigenetic age analysis indicated an average epigenetic “age advancement” (EAA) of 5.4 years (SD 6.6). Median MoCA score was 24 (21-27) and 67% were pre-frail or frail. There were 17 deaths (10.7% mortality rate) from September 2016-September 2023. EAA was inversely related to chronological age (β= -0.31 [95% CI: -0.48, -0.14] (p<0.01) and was associated with lower MoCA T-cell count (β= -0.84 [95% CI: -1.14, -0.53] for every 100 CD4+ T-cells (p<0.01)) in an unadjusted linear regression model. EAA was associated with lower MoCA score in a linear regression model adjusted for age, sex and race (p<0.01), and there was a trend towards EAA association with more advanced frailty state in an ordinal logistic regression model adjusted for age, sex and race (p=0.07). Survival differed by EAA, and greater advancement was associated with 7-year mortality in a Cox Proportional Hazard model adjusted for age (HR 1.10 [95%CI: 1.02, 1.18] p=0.01) (Figure 1).

**Conclusion:** In OPWH the average EAA was 5.4 years, as calculated by PhenoAge, and was associated with cognitive dysfunction, more advanced frailty state, as well as 7-year mortality. These results suggest epigenetic clocks are a valuable biomarker of aging-related pathologies in OPWH and warrant further study.
depressive symptoms (BPSP2), mild-to-moderate NCI with severe depressive symptoms and iADL dependence (BPSP3), and mild-to-moderate NCI without depressive symptoms or iADL dependence (BPSP4). Proteins were quantified by the Olink Target-96 inflammation panel. Linear regression of normalized protein expression values analyzed group differences. Benjamini-Hochberg adjusted p-values were 0.003. Proteins with p<0.10 were next analyzed by multivariable regression adjusting for demographic, disease, and treatment characteristics. Classification accuracy was assessed by discriminant analysis. Results: Cohort characteristics: mean age 45.0 years, 22.8% were women, 51.5% were Black, median CD4+ T-cells 480 µl, median duration: HIV 10.6 years, ART 5.7 years. An example volcano plot is shown in the Figure A. Across all dimensions, the most frequently associated proteins were CD8 antigen, IL-12, IL-17C, FGF-21, FGF-23, and TGF-α. Adjusted multivariable models with R2 >0.15 were found for global NCI, motor impairment, BDHI II somatic subscale, and all BPSPs (Table B). Across all dimensions, discriminant analysis correctly classified >75% of participants in only BPSP3 and BPSP4. The BPSP3 model included CCL19 and DNER and the BPSP4 model included CD8 antigen, IL-33, CCL3, TNFSF11, and stem cell factor.

Conclusion: Data-driven, cross-sectional analyses identified plasma proteins associated with multiple neuropsychiatric dimensions. While the results reinforce the complexity of neuropsychiatric disorders, novel, biologically plausible targets were identified for validation and future investigation. The BPSPs generally performed better in analyses, suggesting that they may be more inflammation-driven than other dimensions. The focus on inflammation is a limitation; future analyses that include other proteins (e.g., neuronal) should provide additional insights.

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### 598 **High Plasma GFAP in Older PWH With Low Nadir CD4 Supports Legacy Brain Injury and Reactive Gliosis**

**Martina Strano**, Federica Marmondi, Sara Diotallevi, Simona Bossolasco, Angelo Roberto Raccagni, Antonella Castagna, Gabriella Scarlatti, Matteo Bonato, Paola Cinque

1San Raffaele Vita-Salute University, Milan, Italy, 2San Raffaele Scientific Institute, Milan, Italy, 3University of Milan, Milan, Italy

**Background:** Measuring cerebrospinal or plasma levels of brain injury markers may provide useful information on HIV neuropahtogenesis and help characterize cognitive impairment in PWH. We compared plasma levels of these markers to cognitive function, physical performance and history of HIV in PWH≥50 years.

**Methods:** 102 ART-treated PWH ≥50 years and 40 age-matched controls without HIV infection were prospectively assessed for Neurofilament Light Chain (NFL), Gial Fibrillar Acid Protein (GFAP), Tau and Ubiquitin C-terminal hydrolase-L1 (UCH-L1) by Simoa Human Neurology 4-plex. PWH were evaluated for neurocognitive performance (TMT-A, TMT-B, Digit Symbol (DS)), depression and anxiety (Hospital Anxiety and Depression scale), physical function (handgrip, chair-stand test for muscle strength, mini-BEST test for balance and mobility), current and nadir CD4+ counts and years of HIV infection. Values were shown as median (IQR); group comparisons were analyzed by Mann Whitney U test and correlations by Spearman’s correlation test and linear regression.

**Results:** No significant differences between PWH and controls were observed for age [60 (57–63) vs. 57 (52–62)] and any of the four markers [NFL: 12.3 (8.2–16.5) vs. 10.6 pg/µl (9.4–14.3); GFAP: 108.3 (76.2–144.2) vs. 95.4 (76.3–138.7); Tau: 2.69 (1.88–4.30) vs. 3.30 pg/µl (2.24–4.89); UCH-L1: 17.9 (10.6–28.8) vs. 19.5 pg/µl (11.4–34.2)]. In PWH, NFL and GFAP were highly correlated (p=0.0004, r=0.356); higher NFL and GFAP correlated to older age (both p<0.0001, r=0.356 and 0.496, respectively) and GFAP levels were higher in women (p=0.01); higher NFL was associated with lower performance at mini-BEST test (p<0.0001, r=0.334), and higher GFAP with lower performance at DS (p=0.043, r=-0.201), handgrip (p=0.0001, r=-0.367) and mini-BEST test (p=0.015, r=-0.239), lower nadir CD4+ counts (p=0.0004, r=0.343) and longer duration of infection (p=0.045, r=-0.199). By multivariate analysis (Table), higher NFL levels were associated with older age and better performance at the Mini-BEST test; higher GFAP with older age, female gender and low nadir CD4+ count.

**Conclusion:** Beyond the expected correlation of higher GFAP and NFL with age, the specific association of higher GFAP level with low nadir CD4+ counts and long infection duration may reflect a legacy injury with reactive gliosis, of a previous untreated CNS HIV infection. Practically, physical performance may help define the functional status associated with elevated markers of CNS cell injury.

<table>
<thead>
<tr>
<th>Variable</th>
<th>NFL Estimate [95% CI]</th>
<th>P value</th>
<th>GFAP Estimate [95% CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>0.00</td>
<td>-0.68 (0.31, 1.65)</td>
<td>0.62</td>
<td>26.28 (26.00, 26.56)</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>0.09 (0.06, 0.11)</td>
<td>0.99</td>
<td>4.44 (4.27, 4.61)</td>
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<tr>
<td>Digit Symbol (score)</td>
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<td>0.06 (0.03, 0.09)</td>
<td>0.64</td>
<td>0.64 (0.62, 0.66)</td>
</tr>
<tr>
<td>Mini-BEST test (score)</td>
<td>0.00</td>
<td>-0.08 (-0.17, 0.01)</td>
<td>0.95</td>
<td>5.98 (5.49, 6.47)</td>
</tr>
</tbody>
</table>

### 599 **Pattern of Age-Related Cognitive Decline in People With HIV Depends on Viral Suppression**

**James T. Kennedy**, Sarah Cooley, Beau Ances

Washington University St. Louis, St. Louis, MO, USA

**Background:** The introduction of combination anti-retroviral therapy (cART) has led to increased life expectancy of persons living with HIV (PWH) and a reduction in the prevalence of HIV-associated neurocognitive disorder. As PWH grow older, it is important to understand the cognitive trajectories of PWH compared to people without HIV (PWnH) and how treatment efficacy impacts age related cognitive changes.

**Methods:** PWnH and PWH (viraemia undetectable, viral load ≤50/mL, PWnH; and detectable; PWnH) were recruited and repeatedly administered a simple cognitive battery — Category Fluency (language), Hopkins Verbal Learning Test (learning subtests), Trails A (psychomotor/processing speed), and Trails B (executive function) — that were combined to form a composite (NP24). The composite was formed by normalizing scores to baseline performance (flipping timed tests so that lower values means poorer performance), and averaging

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**Table:** Data coefficients of the multivariable linear model of NFL and GFAP

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**Pattern of Age-Related Cognitive Decline in People With HIV Depends on Viral Suppression**

**James T. Kennedy**, Sarah Cooley, Beau Ances

Washington University St. Louis, St. Louis, MO, USA

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Z-scores. The association between cognition and age was assessed for each group in a generalized additive mixed model. Education, ethnicity, sex, and time since first cognitive test were included as covariates.

**Results:** A total of 679 participants (279 PWoH, mean [standard deviation] age 40 (17) years old; 305 PWHU, age 49 (14) years old; 95 PWHD, age 40 (17) years old), tested up to 6 times for a total of 968 data points, were analyzed. Age was associated with cognition in each group, with cognition worsening with increasing age. The overall effect significantly differed between all groups (PWoH > PWHU > PWHD). The shape of the age–cognition relationship was identical for PWoH and PWHU, only significantly shifted downward. Shape significantly differed in PWHD, resulting in a steeper, steadier decline in cognition with increasing age relative to PWoH and PWHU.

**Conclusion:** While there is a decrease in cognition associated with HIV, the age–cognition relationship mirrors PWoH when virologically controlled. Older, uncontrolled PWH are at greater risk for cognitive decline and should be the focus of future studies.

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**600 Hepcidin Modifies Effects of Age and Erythrocyte Indices on Cognitive Function in People With HIV**

Azin Tavasoli,1 Olawumiwa K. Okwo-Obagbona, Jennifer E. Iudicello,1 Asha Kallianpur,1 Ronald J. Ellis,1 Scott L. Letendre2

1University of California San Diego, La Jolla, CA, USA, 2Khure Health, Toronto, Canada, 3Case Western Reserve University, Cleveland, OH, USA

**Background:** Cognitive impairment (CI) in people with HIV (PWH) is associated with abnormal erythrocyte indices, which may result from inflammation, disturbed iron metabolism, and other factors. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are indices of erythrocyte volume and hemoglobinization, which help to classify anemia. Erythropoiesis requires bioavailable iron, levels of which are regulated by a complex system of peptide hormones, including the acute–phase peptide, hepcidin.

**Methods:** Iron biomarkers (hepcidin, erythropoietin, ferritin, erythrophorene, total iron-binding capacity, soluble transferrin receptor) and erythrocyte indices were quantified using commercial assays in 88 virally suppressed antiretroviral therapy (ART) treated PWH. All participants underwent comprehensive cognitive testing, and their performance was summarized by T-scores. Multivariable regression analysis of cognitive performance was performed using the Akaike Information Criterion and backward selection.

**Results:** Participants were mostly middle-aged (mean age 44 years), white (52.3%), and men (84.1%). Hepcidin levels were detectable (>1.0 ng/mL) in 42 (47.7%) and modified the relationship between global T-score and either age (interaction p-value=0.0072) or MCV (interaction p-value=0.0119) or MCH (p=0.0127). Stratified analyses identified that lower global T-scores were associated with older age (p=0.001) or higher MCV (p=0.0046) only when hepcidin was undetectable. A similar analysis was performed to assess interactions between anemia and iron indices in relation to global T-scores and this identified statistically significant interactions between anemia and either MCV (p=0.0119) or MCH (p=0.0127). Stratified analyses identified that lower global T-scores were associated with higher MCV (p=0.0015), and higher MCH (p=0.0036) only among anemic participants. These associations remained statistically significant after adjustment for sex, race, ethnicity, duration of HIV infection, CD4+ T-cell count and nadir, duration of ART, and HCV serostatus.

**Conclusion:** These findings suggest that a combination of iron-related (hepcidin, MCH) and iron-unrelated (age, higher MCV) mechanisms influence cognitive health. While these cross-sectional results require confirmation in larger, longitudinal studies with more women, they highlight hepcidin as a potential modifying factor in associations of age and erythrocyte indices in relation to cognitive performance in PWH.

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**601 VACS 2.0 Shows Improved Discrimination of Neurocognitive Impairment and Frailty in People With HIV**

Cynthia Yan, Sarah Cooley, Beau Ances

Washington University in St Louis, St Louis, MO, USA

**Background:** The Veterans Aging Cohort Study (VACS) 1.0 Index is a generalizable risk index that combines and weights age, CD4 count and human immunodeficiency virus (HIV) type 1 RNA alone (Restricted Index), hemoglobin, FIB-4 Index, hepatitis C virus, and estimated glomerular filtration rate. The VACS 1.0 index more accurately discriminates mortality risk among persons with HIV (PWH) prescribed antiretroviral therapy than traditional HIV markers and age alone. More recently, there have been revisions to VACS 1.0 index. This study examined whether the updated VACS 2.0 index (including serum albumin, body mass index (BMI), and white blood cell (WBC) count) had stronger correlations with cognitive function, brain volume, and frailty in older (≥50 years) PWH compared to the original VACS 1.0.

**Methods:** Neuropsychological performance (NP) Z-scores (learning, retention, executive functioning (EF), psychomotor functioning/processing speed (PM/PS), language, and global cognition), and neuroimaging measures (brain volumetrics) were analyzed in PWH (n=162, 88.17% male). A subset of the sample (n = 159) was defined as either frail (n = 18) or non-frail (n = 141) according to the Fried phenotype criteria. Brain volumes, NP scores, and frailty subgroups were analyzed with both VACS scores, albumin, BMI, and WBC count using Pearson’s significance tests and independent T-tests.

**Results:** Based on values shown in Table 1, higher VACS scores significantly correlated with lower brain volumes. A higher VACS 2.0 score was associated with lower NP in the EF and PM/PS domains and was primarily driven by albumin. In contrast, VACS 1.0 scores did not correlate with cognition Z-scores. There was no relationship between frailty status and VACS 1.0. PWoH who were frail had significantly greater VACS 2.0 scores than non-frail PWH.

**Conclusion:** The addition of serum albumin to the VACS index improved its correlations with NP and frailty in PWH. While low albumin levels may contribute to cognitive decline or frailty, the reverse causality should also be considered. For example, those with frailty or cognitive impairment may struggle to maintain proper nutrition, potentially resulting in decreased albumin levels. Regardless of the direction of causality, these findings suggest that the VACS 2.0 index and albumin are valuable measures for clinicians to improve outcomes in older PWH.

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**602 Latent Viral Infections Are Associated With Veterans Aging Cohort Study Index in People With HIV**

Patricia K. Riggs, Gordon Honerkamp Smith, Milenka Meneses, Antoine Chaillon, Gemma Caballero, Donald Franklin, Robert K. Heaton, Ronald J. Ellis, Scott L. Letendre, Sara Gianella Weibel

University of California San Diego, San Diego, CA, USA

**Background:** People with HIV (PWH) on ART have elevated risk for mortality, frailty, and cognitive impairment. The Veterans Aging Cohort Study (VACS) index is a validated risk score associated with these outcomes in PWH. CMV and EBV coinfections have been implicated in these outcomes and expansion of the HIV reservoir but it is unknown if this is a direct effect of the virus or the immune response. We sought to determine the relationships among quantitative cell associated viral DNA, IgG levels, and VACS index score.

**Methods:** Participants included 485 PWH with comprehensive neuromedical testing. HIV RNA<200 copies/mL on antiretroviral therapy, and stored blood. Digital droplet PCR quantified cell associated CMV, EBV, and HIV DNA in peripheral blood mononuclear cells (PBMCs). EBV VCA IgG and CMV IgG were measured in plasma by ELISA. Using linear regression, we tested the associations of quantitative cell associated viral DNA, IgG levels, and VACS index score.

**Results:** The addition of serum albumin to the VACS index improved its correlations with NP and frailty in PWH. While low albumin levels may contribute to cognitive decline or frailty, the reverse causality should also be considered. For example, those with frailty or cognitive impairment may struggle to maintain proper nutrition, potentially resulting in decreased albumin levels. Regardless of the direction of causality, these findings suggest that the VACS 2.0 index and albumin are valuable measures for clinicians to improve outcomes in older PWH.

**Table 1: Correlations of Brain Volume and Cognitive Z-Scores with VACS Indices and Serum Albumin**

<table>
<thead>
<tr>
<th>Domain</th>
<th>VACS 1.0 Scores</th>
<th>VACS 2.0 Scores</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Total Gray Matter</td>
<td>-0.060494</td>
<td>0.089917</td>
<td>-0.316793</td>
</tr>
<tr>
<td>Executive Function</td>
<td>-0.038545</td>
<td>0.176127</td>
<td>0.020797</td>
</tr>
<tr>
<td>Performance Function/Processing Speed</td>
<td>0.150358</td>
<td>0.000147</td>
<td>-0.160984</td>
</tr>
<tr>
<td>General Cognitive Function</td>
<td>-0.032512</td>
<td>0.250498</td>
<td>0.153776</td>
</tr>
</tbody>
</table>

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**Conclusion:** The addition of serum albumin to the VACS index improved its correlations with NP and frailty in PWH. While low albumin levels may contribute to cognitive decline or frailty, the reverse causality should also be considered. For example, those with frailty or cognitive impairment may struggle to maintain proper nutrition, potentially resulting in decreased albumin levels. Regardless of the direction of causality, these findings suggest that the VACS 2.0 index and albumin are valuable measures for clinicians to improve outcomes in older PWH.
Background: HIV-associated neurocognitive disorder (HAND) is a range of progressively severe central nervous system complications associated with HIV infection. The range can be from mild problems with memory, language, and reasoning to the more severe HIV-associated dementia. HAND has declined with the advancement of antiretroviral therapy (ART), however, remains prevalent in older people with HIV (PWH). We describe the prevalence of HAND among PWH in the US.

Methods: We analyzed data from Truven Health MarketScan Claims and Encounters®, a large database derived from administrative claims for healthcare services provided to Medicaid enrollees. Among enrolled persons aged ≥18 years, we identified PWH with at least one inpatient or outpatient medical claim with an HIV and subsequent major to mild neurocognitive disorders (NDs) ICD-10-CM diagnosis codes. We used chi-square to compare PWH with an associated NDs diagnosis (HAND) to those without an associated ND diagnosis code (no-HAND) to investigate differences in HAND prevalence by age, sex, race/ethnicity, and by prescribed ART from 2016-2021.

Results: The annual crude prevalence of HAND ranged from 1.6% (512/31,897) in 2016 to 1.9% (558/29,967) in 2020. The prevalence of HAND significantly increased with age (p-value<0.001); highest prevalence was among ages 65+ years (range 3.7% in 2021;5.7% in 2017). Males had a significantly higher prevalence of HAND compared to females in 2016 (1.8% vs 1.4%; p-value=0.007) and 2017 (1.5% vs 1.6%; p-value=0.027) but from 2018-2021 there were no differences by sex. In 2016 and 2020 there were differences by race/ethnicity (p-value=0.0002 and 0.001 respectively), with the highest prevalence of HAND among White persons in 2016 (1.9%) and Black persons in 2020 (2.1%). Among the 190,648 PWH from 2016-2021, the frequency of ART prescriptions ranged from 58% (18,671/31,897) in 2016 to 64% (21,018/32,820) in 2021. Prevalence of HAND was significantly higher among persons without an ART prescription (range 1.8% in 2016-2.2% in 2020) compared to those with an ART prescription (range 1.1% in 2021-1.7% in 2020) for each year (Figure).

Conclusion: In this large administrative database of PWH, HAND remains persistent, with some demographic disparities. With the aging population of PWH, strengthening interventions that improve access to ART, promote adherence, and address barriers to clinical care and supportive services for PWH is critical for reducing health disparities.
**Evidence for Safe Use of Doravirine With Hormonal Contraceptives in African Women Living With HIV**

Rena Patel,1 Nikosi Ndlove2,1, La-Donna Kapa3, Garams Basha4, Ché K. Moshesh5, Nashon Yongo6, Krishnaveni Reddy3, Hompeumeleo Sigau1, Nazmeen Cassim1, Shuki A. Hassan2, David W. Erikson2, Kimberly K. Scarsi2, Thesha Palanese-Phillips5, for the EPIC Study Team.

**Background:** Prior to scale-up of new ART, exploring drug-drug interactions between ART and contraceptive methods is crucial. We are conducting EPIC, a parallel group pharmacokinetic study to examine interactions with doravirine (DOR)-containing ART among women of reproductive potential living with HIV in Johannesburg, South Africa.

**Methods:** EPIC began in November 2021 and is ongoing. A ≥6-week oral lead-in period was required after antiretroviral participants switched from their existing ART to DOR-containing ART. Participants in the 3 intervention groups self-selected concomitant contraception: intramuscular depot-medroxyprogesterone acetate (IM DMPA; Group 1), etonogestrel implant (ETG; Group 2), or copper intrauterine device (IUD; Group 3). Comparator groups included a contemporaneous doravirine (DTG) + IM DMPA (Group 4) and a historical DTG + ETG implant (Group 5). Participants were followed up for 12 weeks (Groups 1 & 4) or 24 weeks (Groups 2, 3, & 5) for PK sampling. We did not detect significant effects of DOR-containing ART on safety (frequency, severity of adverse events) and tolerability (patient satisfaction, adherence) of DOR-containing ART.

**Results:** A total of 108 Black African women were enrolled (Table 1): Group 1 (n=21), Group 2 (n=23), Group 3 (n=19), Group 4 (n=21), and Group 5 (n=24). GM Cmin MPA concentrations for Groups 1 and 4 were 561 and 551 pg/mL, respectively (GMR 1.15, 90% CI 1.04, 1.28; adjusted GMR 1.16, 90% CI 1.04, 1.30).

**Conclusion:** We did not detect significant effects of DOR-containing ART on MPA or ETG contraceptives. DOR-containing ART appears to be safe and tolerable. 2 (3%) participants reported any dissatisfaction with this ART and adherence by pill count was 80% for >76% adherence.

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**Table 1: Baseline characteristics of and pharmacokinetic results among African women living with HIV. EPIC study (n=108), Nov 2021-Mar 2022.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 (n=21)</th>
<th>Group 2 (n=23)</th>
<th>Group 3 (n=19)</th>
<th>Group 4 (n=21)</th>
<th>Group 5 (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.9 (32.1, 37.5)</td>
<td>34.6 (31.1, 37.5)</td>
<td>34.2 (30.4, 37.5)</td>
<td>34.9 (32.1, 37.5)</td>
<td>34.6 (31.1, 37.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 (23.0, 27.1)</td>
<td>25.6 (23.0, 27.8)</td>
<td>24.9 (22.4, 27.4)</td>
<td>25.1 (23.0, 27.1)</td>
<td>25.6 (23.0, 27.8)</td>
</tr>
<tr>
<td>Income ($)</td>
<td>220 (180, 260)</td>
<td>225 (190, 270)</td>
<td>220 (180, 260)</td>
<td>225 (190, 270)</td>
<td>220 (180, 260)</td>
</tr>
<tr>
<td>Education</td>
<td>12 (11, 13)</td>
<td>12 (11, 13)</td>
<td>12 (11, 13)</td>
<td>12 (11, 13)</td>
<td>12 (11, 13)</td>
</tr>
</tbody>
</table>

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**Pharmacokinetic results for contraceptive hormones (ng/mL) for each group.**

- **MPA**
  - DMPA (Group 1): 42.1 (35.1, 49.2) ng/mL
  - ETG (Group 2): 34.3 (29.7, 39.6) ng/mL
  - IUD (Group 3): Data not provided
  - Group 4: 42.1 (35.1, 49.2) ng/mL
  - Group 5: 42.1 (35.1, 49.2) ng/mL

- **ETG**
  - DMPA (Group 1): 53.2 (48.2, 58.3) ng/mL
  - ETG (Group 2): 49.8 (45.3, 54.2) ng/mL
  - IUD (Group 3): Data not provided
  - Group 4: 53.2 (48.2, 58.3) ng/mL
  - Group 5: 53.2 (48.2, 58.3) ng/mL

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**Conclusion:** We did not detect significant effects of DOR-containing ART on MPA or ETG contraceptives. DOR-containing ART appears to be safe and tolerable, and findings support the viability of this option for those living in resource-limited settings.
Population Pharmacokinetic Approaches to Standardize Antiviral Exposure in the Cerebrospinal Fluid

Sean N. Aredussian1, Yimg Mu1, Caitlyn McCarthy1, Ronald J. Bosch1, Rajesh T. Gandhi2, Deborah K. Mahanor1, Joseph J. Eron1, John W. Mellors2, CourtneYV. Fletcher1

1University of Nebraska Medical Center, Omaha, NE, USA; 2Harvard Th Chan School of Public Health, Boston, MA, USA

Background: HIV has been shown to persist in the central nervous system (CNS) in persons on antiretroviral therapy (ART). Consequently, CNS persistence may be linked to inadequate ART exposure. When assessing CNS drug levels in participants on ART, it is difficult to estimate drug exposure given sparse sampling and to standardize exposure given different sampling times among participants. We describe pharmacokinetic (PK) methods to estimate CNS exposure (maximum concentration [Cmax], area under the curve [AUC], and trough [Ctrough]) among individuals that allows a standardized evaluation of CNS drug exposure.

Methods: AS321 is a prospective study of HIV-1 reservoirs among persons with HIV on long-term virologically-suppressive ART. 59 participants had plasma and cerebrospinal fluid (CSF) concentrations measured ranging from 1 to 23 hrs post ART dose. Population PK modeling was performed for FTC, TDF, EFV, 3TC, ATV/r, RAL, DTG, DRV/r, and EVG. The simplest PK model of plasma and CSF was considered for each ARV utilizing Pmetrics (version 1.5.0; Los Angeles, CA) for R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). The final PK model was used to obtain predicted plasma and CSF estimates at 12-minute intervals from each participant’s measured ARV plasma and CSF concentrations. Noncompartmental analysis was used to calculate AUC. Relative CNS penetration for each ARV was estimated by comparing CSF Cmax and AUC to plasma Cmax and AUC (i.e., relative CNS penetration= Cmax-CSF / Cmax-Plasma and AUCCSF / AUC of each ARV.)

Results: Models converged for a combined plasma and CSF 3-compartment oral absorption model. FTC exhibited the highest median CSF penetration (Cmax, 46%; AUC,72%). The lowest median penetration was observed for both DRV/r (Cmax, 0.95%; AUC,1%) and DTG (Cmax, 0.57%; AUC,0.57%). All ARVs had median CSF Ctrough concentrations > IC50 except TFV: Ctrough<0.016mg/L < IC50,0.1473mg/L.

Conclusion: These methods demonstrate an approach of utilizing PK modeling to standardize drug levels to a given time point (i.e., Cmax or Ctrough) and assess if desired therapeutic drug goals are obtainable in the CNS. Further studies are warranted to address whether CNS exposure as calculated using this method is associated with measures of HIV persistence in the CNS.

Table 1. Antiretroviral exposure summary of 59 participants

<table>
<thead>
<tr>
<th>ARV</th>
<th>Cmax Median (IQR)</th>
<th>AUC Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTC</td>
<td>46% (24-84%)</td>
<td>72% (13-100%)</td>
</tr>
<tr>
<td>TDF</td>
<td>0.95% (0.05-2.81%)</td>
<td>1% (0.005-0.48%)</td>
</tr>
<tr>
<td>EFV</td>
<td>0.57% (0.05-4.28%)</td>
<td>0.57% (0.005-0.48%)</td>
</tr>
<tr>
<td>3TC</td>
<td>0.05% (0.005-0.48%)</td>
<td>0.05% (0.005-0.48%)</td>
</tr>
<tr>
<td>ATV/r</td>
<td>0.05% (0.005-0.48%)</td>
<td>0.05% (0.005-0.48%)</td>
</tr>
<tr>
<td>RAL</td>
<td>0.05% (0.005-0.48%)</td>
<td>0.05% (0.005-0.48%)</td>
</tr>
<tr>
<td>DTG</td>
<td>0.05% (0.005-0.48%)</td>
<td>0.05% (0.005-0.48%)</td>
</tr>
<tr>
<td>DRV/r</td>
<td>0.95% (0.05-2.81%)</td>
<td>1% (0.005-0.48%)</td>
</tr>
</tbody>
</table>

Tenofivir Diphosphate Benchmarks in Dried Blood Spots in PWH Receiving TAF-Based ART (QUANTI-TAF)

Ryan P. Coyle1, Mary Morrow1, Vincent Mainella1, Sarah C. Mann1, Nicholas Barker1, Lucas Ellison1, Samuel L. Ellis1, Pamela A. Alpert1, Tony C. Carnes2, D. Eric Buffkin1, Lane Bushman1, Samantha MalWhinney1, Kristina M. Brooks1, Jose R. Castillo-Mancilla1, Peter L. Anderson1

1University of Colorado Anschutz Medical Campus, Aurora, CO, USA; 2etectRx, Gainesville, FL, USA

Background: Tenofivir diphosphate (TFV-DP) concentrations in dried blood spots (DBS) in people with HIV (PWH) receiving tenofivir alafenamide (TAF)-based antiretroviral therapy (ART) are not defined. QUANTI-TAF (NCT04065347) aimed to establish adherence/concentration benchmarks in PWH receiving TAF-based ART by quantifying TFV-DP concentrations in DBS, leveraging digital pills to organically capture directly observed therapy (DOT) adherence in a real-world clinical cohort.

Methods: PWH receiving TAF-based ART for ≥6 months were prospectively enrolled in a 16-week pharmacokinetic study. Digital pills captured DOT adherence by co-encapsulation of each TAF-based ART dose with an ingestible biosensor powered by gastric fluid. Blood for TFV-DP concentrations in DBS (fmmol/2x7mm punches) was collected at each visit and quantified using a validated LC-MS/MS method. TFV-DP steady-state concentrations (Css) in DBS were assessed at weeks ≥12 overall, by DOT adherence, booster status, and HIV viral load (VL; all weeks), reported as median (IQR).

Results: N=64 participants (median age 54 [43, 59] years) receiving TAF for median 32 (18, 45) months were enrolled. Most were males (89%) receiving we present a map of ARVs penetration across different post-mortem tissue homogenates from altruistic participants recruited in the Last Gift program. Methods: People with HIV with terminal illness who gave written informed consent were enrolled. All participants were on suppressive ART until the time of death. Tissue samples were collected through rapid research autopsy (<6h after death) and immediately snap frozen in liquid nitrogen. Non-nucleoside ARVs intra-tissue concentrations were determined through an UHPLC MS/MS validated method, with accuracy and precision within the requirements of FDA guidelines, after homogenization of 2 aliquots (about 30 mg) of tissue for each sample. The results were normalized by weight and the mean values were reported as results.

Results: 21 tissue biopsies per participant were isolated from different anatomical sites in 6 volunteers on different ART regimens: 3 on tenofovir alafenamide/emtricitabine (TAF/FTC) and dolutegravir (DTG), 1 on TAF/ FTC+DTG plus rilpivirine (RPV), 1 on DTG and darunavir/cobicitabist (DRV/C) and 1 on abacavir/lamivudine (ABC/3TC)/DTG. DTG had a higher exposure in intestine, kidney, and liver and lower in central nervous system (CNS) and pancreas (p=0.001 and p=0.012 compared to intestine, respectively). DRV, RPV and cobicitabist (COBI), in a lower sample size (1 participant/drug), showed a higher exposure in liver and intestine, compared with other anatomical sites as reported in Fig. 1. No statistical differences were observed in the overall ARVs penetration in different areas in the brain (p=0.971) and intestine tracts (p=0.941).

Conclusion: Scarce data on human intra-tissue ARV penetration are reported in literature. This is the first study to report different ARV concentrations in different anatomical sites in humans. Previous studies on non-human primates showed similar results about the poor, but rather uniform, concentrations of DTG in all the districts of the CNS, and higher concentrations in kidney and intestine.

Antiretroviral Concentrations in Post-Mortem Tissues: Preliminary Results From the Last Gift Program

Micol Ferrara1, Amedeo De Nicolò1, Alessandra Manca1, Elisa De Vivo1, Sara Soloperto1, Davey M. Smith1, Antoine Chaillon1, Magali Porrachia1, Nicham Higgins1, Antonia D’Avolio1, Stefano Bonora1, Sara Gianella Weibel1

1University of Turin, Turin, Italy; 2University of California San Diego, San Diego, CA, USA

Background: Antiretroviral therapy (ART) successfully inhibits HIV replication but cannot eradicate the viral reservoir in various anatomic compartments. This is partially due to differential antiretroviral (ARV) drug penetration in different compartments. In humans, ARVs measurement in reservoirs is complicated by technical and ethical obstacles in performing tissue biopsies in vivo. Here
integrate strand transfer inhibitors (88%). Race/ethnicity was 55% White, 24% Black, and 21% Hispanic/Latino. TFV-DPCss in DBS (n=77) were higher than PBMC. PWH with ≥85% DOT adherence (n=6) had median 2998 (2152, 3610) fmol/punches; PWH with >85% DOT adherence (n=69) had median 3339 (2629, 4378) fmol/punches (A). TFV-DP Css in DBS were higher with boosted (b/) than unboosted (un) ART. PWH receiving an ART (n=72) had median 3274 (2542, 4323) fmol/punches; PWH receiving b/ART (n=5) had median 8210 (5893, 8760) fmol/punches (B). No HIV VL was >200 cps/mL. Low-level viremia (LLV) occurred at 60/535 (10%) visits from 13/24 (54%) participants (VL range: 20-149 cps/mL), with similar TFV-DP concentrations in DBS: median 3176 (2949, 4145) fmol/punches at LLV visits, median 3278 (2585, 4404) fmol/punches at suppressed (VL <200 cps/mL) visits. Digital pills were well tolerated.

**Conclusion:** We describe initial TFV-DP benchmarks in DBS in PWH receiving TAF-based ART as digital pills to capture DOT adherence in a real-world clinical study. We observed higher TFV-DP Css with b/ART than un/ART. The lack of relationship between LLV and TFV-DP concentrations in DBS in this cohort suggests mechanisms other than variable adherence for LLV. Future analyses will explore additional factors that may be associated with LLV.

### 610 Factors Affecting TFV-DP Concentrations in PBMC and Relationships With DBS in PWH on TAF-Based ART

**Stefanie Schwab, MD, Mary Morrow, MD, Corwin Copping, MD, Ryan P. Coyte, MD, Vincent Mainella, MD, Sarah C. Mann, MD, Nicholas Barker, MD, Lucas Ellison, MD, Samuel L. Ellis, MD, Pamela E. Alpert, MD, Lane Bushman, MD, Samantha MalWhitney, MD, Kristina M. Brooks, MD, Jose R. Castillo-Mancilla, MD, Peter L. Anderson, MD**

**University of Colorado Anschutz Medical Campus, Aurora, CO, USA**

**Background:** Relationships between tenofovir-diphosphate (TFV-DP) steady-state concentrations (Css) in peripheral blood mononuclear cells (PBMCs), dried blood spots (DBS), and adherence have not been well-described in people with HIV (PWH) on tenofovir-alafenamide (TAF)-containing antiretroviral therapy (ART). Our objectives were to assess the influence of adherence and other factors on TFV-DP Css in PBMCs, and to examine relationships between TFV-DP Css in DBS and PBMC.

**Methods:** QUANTI-TAF was a 16-week observational study that enrolled PWH on TAF-based ART for >6 months. Study visits occurred at weeks 0, 4, 8, 12, and 16. Blood was collected for TFV-DP in DBS (2x7mm punches) and PBMC (per 106 cells) at each visit and quantified using validated LC-MS/MS methods. Adherence was quantified using TAF-based ART co-processed with an ingestible biosensor (calculated as # ingestions in the 10 days before each visit).

Week 4 TFV-DP Css in PBMC were dichotomized by 5-8 vs. 9-10 days of dosing (Fig. A). Median TFV-DP Css of boosted ART patients was 3.00 (IQR: 2.25 – 3.75) µg/mL at 1 day after dosing, while unboosted ART patients had median 2.00 (IQR: 1.50 – 2.50) µg/mL. Week 4 TFV-DP Css for b/ART was 3.75 (IQR: 3.00 – 4.50) µg/mL, which was significantly higher than un/ART (3.00 µg/mL). Median TFV-DP Css for 9-10 vs. 5-8 days of dosing (Fig. B). No HIV VL was >200 cps/mL. Low-level viremia (LLV) occurred at 60/535 (10%) visits from 13/24 (54%) participants (VL range: 20-149 cps/mL), with similar TFV-DP concentrations in DBS: median 3176 (2949, 4145) fmol/punches at LLV visits, median 3278 (2585, 4404) fmol/punches at suppressed (VL <200 cps/mL) visits. Digital pills were well tolerated.

**Conclusion:** We describe initial TFV-DP benchmarks in DBS in PWH receiving TAF-based ART as digital pills to capture DOT adherence in a real-world clinical study. We observed higher TFV-DP Css with b/ART than un/ART. The lack of relationship between LLV and TFV-DP concentrations in DBS in this cohort suggests mechanisms other than variable adherence for LLV. Future analyses will explore additional factors that may be associated with LLV.

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**611 Adherence Markers for Doxy-PEP in Plasma and Urine**

**Richard Haaland, MD, Jeffrey Fountani, MD, Chuong Dinh, MD, Tiancheng Edwards, MD, Deborah Omoye, MD, Christopher Conway-Washington, MD, Colleen Kelley, MD, Wald Heneine, MD**

**1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Emory Vaccine Centre, Atlanta, GA, USA, 3Emory AIDS Research, Atlanta, GA, USA**

**Background:** Clinical trials of Doxy-PEP demonstrated high efficacy in preventing bacterial sexually transmitted infections (STIs) among men who have sex with men but not in women. Low Doxy-PEP efficacy observed among women may be related to poor adherence highlighting the importance of objective adherence monitoring in future trials and implementation studies.

We examined doxycycline (DOXY) concentrations in urine and plasma, two specimen types commonly collected for STI testing, following a single oral DOXY dose to identify objective adherence markers of Doxy-PEP dosing.

**Methods:** Eleven male and 9 female participants provided blood and urine up to 7 days after receiving a 200 mg oral DOXY dose. DOXY was measured in plasma and urine by liquid chromatography-mass spectrometry with a lower limit of quantification of 10 ng/mL. DOXY concentrations are reported as median and interquartile range. Adherence indicators were identified as the 10th percentile concentration at each time point.

**Results:** Plasma DOXY concentrations peaked 2 hours after dosing and declined to 0.487 µg/mL (0.402 – 0.682 µg/mL) 24 hours after dosing. Plasma DOXY remained measurable in all participants 96 hours after dosing (0.043 µg/mL; 0.032 – 0.067 µg/mL) but became undetectable in 13 of 16 participants by 7 days post dose. Urine DOXY concentrations were significantly greater than those in plasma with a urine to plasma ratio of 38.1 (9.1 – 103.3; p < 0.001). Urine DOXY concentrations also peaked 2 hours after dosing and declined to 13.1 µg/mL (6.8 – 28.5 µg/mL) 24 hours after dosing but remained measurable in 15 of 16 participants 7 days after dosing at 0.202 µg/mL (0.137 – 0.318 µg/mL). DOXY adherence indicators for plasma were determined to be 0.30 and 0.03 µg/mL at 1 and 4 days after dosing, respectively, while urine adherence indicators were determined to be 3.00, 0.35 and 0.07 µg/mL at 1, 4 and 7 days after dosing, respectively. Concentrations were not significantly different between male and female participants at each time point.

**Conclusion:** We identified adherence indicators to a single DOXY dose within a pharmacologic tail of 4 and 7 days in plasma and urine, respectively. The data will inform and enable adherence testing in clinical studies to better assess Doxy-PEP efficacy.

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**612 Safety and PK/PD of a Tenofovir Rectal Douche Administered in Different Sequences, DREAM-03**

**Ruohei Zheng, MD, Ken Ho, MD, Edward J. Fuchs, MD, Alex Carballo-Diéguez, MD, Lisa C. Rohan, MD, Rebecca Giguere, MD, Rhonda M. Brand, MD, Stacey Edick, MD, Rahul P. Bakhshi, MD, Teresa L. Parsons, MD, Cindy E. Jacobson, MD, Christina Bagia, MD, Lin Wang, MD, Mark Marzinke, MD, Craig W. Hendrix, MD**

**1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2University of Pittsburgh, Pittsburgh, PA, USA, 3New York State Psychiatric Institute, New York, NY, USA, 4Magee-Womens Research Institute, Pittsburgh, PA, USA**

**Background:** On-demand and behaviorally congruent forms of HIV pre-exposure prophylaxis (PrEP) have long been requested by communities at risk of HIV, especially men who have sex with men (MSM). Previously in DREAM-01, we reported safety, acceptability, and pharmacokinetics/pharmacodynamics (PK/
PD) of three single-dose tenofovir (TFV) rectal douche formulations in MSM and identified a lead formulation. Given that MSM report using multiple douches for cleanliness prior to receptive anal intercourse, DREAM-03 sought to report safety and PK/PD outcomes when using multiple TFV douches with and without water douches. **Methods:** TFV douche products that consisted of 660 mg TFV in 125 mL hypo-osmolar saline were tested in three sequences: (A) three TFV douches, (B) one TFV douche then two tap water douches, and (C) two tap water douches then one TFV douche. We collected blood over 168 hours post-dose and rectal swabs/tissue biopsies over 72 hours. TFV and TFV diphosphate (TFV-DP) concentrations were quantified using validated methods. Anti-HIV effect was evaluated using an ex vivo colonic explant HIV challenge method with biopsies collected over 6 hours post-dose. An HIV p24 assay was used to quantify viral replication. Results among different sequences were compared using Wilcoxon signed-rank tests. **Results:** Nine male participants were enrolled, with a median (range) age of 38 (29, 52) years and a median weight of 77 (64, 113) kg. No grade >2 study related adverse events were reported. Plasma TFV concentrations at 4 and 6 hours were significantly higher (4.9- and 6.5-fold, respectively) in sequence A than those in sequence B (Figure 1A). A trend of higher TFV-DP concentrations in rectal mucosal mononuclear cells (MMCs) at 24 and 72 hours in sequences A (12.0- and 3.5-fold, respectively) and C (5.1- and 4.2-fold, respectively) than those in sequence B were also observed (Figure 1B). Compared to pre-drug baseline, HIV replication after ex vivo HIV challenge demonstrated a concentration-response relationship with 2.8 log₂, 2.2 log₂, and 2.2 log₂ maximal effects for sequences A, B, and C, respectively. **Conclusion:** Our result demonstrates that administering three TFV rectal douches are well tolerated. In addition, using non-medicated douches after a TFV douche may likely reduce both systemic and local TFV exposures, and may subsequently compromise anti-HIV effect of the TFV douche. Our study suggests that after non-medicated douches, a TFV douche should be used to provide better protection against HIV.

Figure 1. (A) TFV concentrations in plasma and (B) TFV-DP concentrations in rectal mucosal mononuclear cells (MMCs) over time. Data is represented as median [interquartile range]. *p<0.05 comparing 4- and 6-hour data between sequences A and B; B is set to =0.05, no rectal MMCs collected at 168 hours.

613 Early Identification of ART Missed Doses: Baseline Data From the RETAIN Study

Lauren Jennings1, Chantel Schreuder1, Richard Madimabe1, Campbell McDuling2, Tebogo Mosina1, Lara Sabin1, Catherine Orrell1
Deshmond Tutu HIV Foundation, Cape Town, South Africa, 1University of Cape Town, Cape Town, South Africa, 2Boston University, Boston, MA, USA

**Background:** We present baseline data from “Improving Retention and viral load outcomes for people taking Antiretroviral therapy through early Identification of missed doses (RETAIIN),” a cohort study exploring detailed adherence metrics (viral load (VL), electronic adherence monitoring (EAM), tenofovir diphosphate (TFV-DP) concentrations and self-report (SR)) in people on ART, when initially flagged for reduced adherence. **Methods:** ART-naive people from three Cape Town ART clinics had adherence monitored by missed doses (EAM), missed clinic visits or by raised VL. At the time of first flagging by any measure, participants received an adherence support call, and were invited for blood draw for HIV-1 VL and TFV-DP (indicating dosing over 4-8 weeks); urine collected for tenofovir rapid assay (indicating dosing over 3-5 days). SR adherence and EAM data were collected for the prior 30 days. Initial adherence data at the time of first non-adherence are presented here. **Results:** Between July 22 and August 23, 116 of 427 (27%) people were flagged for poor ART adherence; 93 (80%) by missed doses, 20 (17%) by missed visits and 3 (3%) by raised viral load. 87 (75%) were women, with mean ±SD age of 28 (±8) years. Median (IQR) self-reported adherence was 90% (83-97) taken in past 30 days; EAM showed reduced adherence across all groups and 3 (3%) by raised viral load. 87 (75%) were women, with mean (±SD) age of 3-5 days). SR adherence and EAM data were collected for the prior 30 days.

614 Baseline Urine Methamphetamine (UTOXM) Predicts ART Adherence and Poor Retention in Care Attributes

Sara Browne1, Anya Umlauf1, Sarah Rojas1, Theodoros Katissias1, Florin Vaida2, Constance A. Benson1
1University of California San Diego, La Jolla, CA, USA, 2Family Health Centers of San Diego, San Diego, CA, USA

**Background:** The CDC estimates 57% of US persons with HIV (PWH) achieve viral suppression. Early initiation of ART is recommended, but objective baseline tests with predictive ability to distinguish persons at risk of poor ART adherence and engagement in care are lacking. We evaluated the utility of baseline urine toxicology testing for methamphetamine (UTOXM) use in PWH starting oral ART in our West Coast US setting to predict adherence and characteristics associated with poor engagement in care. **Methods:** PWH initiating ART with ingestible-sensor-enabled antiretroviral technology (IS-ARVs) to observe medication taking for the first 16 weeks of treatment had a baseline UTOXM with demographics and self-report questionnaires obtained. UTOXM ability to predict IS-ARV-confirms doses (taking & timing); persistence on study; and baseline demographics and questionnaires were analyzed using matched-effects logistic regression; Kaplan-Meier curves; and linear models, respectively. No causal analyses were attempted. **Results:** Sixty-three enrolled participants prescribed IS-ARVs had median age 37 (IQR 30-46) yrs, 82.5% were male, 33.3% White, 34.9% Hispanic, 22.2% African American, 3.2% Asian. UTOXM was negative in 42, positive in 21. Over 6049 observation days evaluated in longitudinal mixed effects logistic regression revealed a negative baseline UTOXM (compared to those who tested positive) was associated with a daily confirmed dose odds ratio (OR) of 12.32 (CI 1.71-7.51), P<0.001 and a regularity of dose timing OR of 5.05 (CL 2.43-10.6), P<0.001. A baseline positive UTOXM (vs negative) was associated with: higher homelessness/ transient housing rate (52.4% vs 21.4%, P=0.028); lower mean income $7,400 vs $31,500, P=0.042; more depression as measured by Mood PHQ 11.8 vs 6.9, P=0.001 and Higher Life Chaos score 14.2 vs 9.0, P=0.001. UTOXM positive 16-week persistence on study was 0.332 (0.102-0.610) vs negative 0.619 (95% CI 0.488-0.785), P=0.0098. **Conclusion:** Baseline positive UTOXM in PWH starting oral ART predicted highly significant differences in treatment adherence & persistence on study (a surrogate for care retention); and identified a distinct subpopulation in our West Coast US setting with multiple attributes, including life chaos & depression, associated with poor care engagement. Baseline UTOXM may be a useful, simple screening tool to identify PWH requiring differentiated care and adherence support on initiation of oral ART, and may be easily employed in mobile testing sites offering quick start care.

Table 1. Early Identification of ART missed doses: baseline data from the RETAIN study.
Preclinical to Human Scaling of Pharmacokinetics for Long-Acting Injectable Antiretrovirals

Henry Pertinez, Rajith Rajoli, Andrew Lloyd, Joanne Sharp, Joanne Herriot, Edyta Kijak, Eduardo Gallardo-Toledo, Megan Neary, Helen Cox, Chloe Bramwell, Anthony Valentijn, Usman Arshad, Paul Curley, Charles W. Flexner, Andrew Owen

University of Liverpool, Liverpool, United Kingdom, The Johns Hopkins University, Baltimore, MD, USA

Background: Long-acting injectables (LAIs) have attracted interest for prevention and treatment of infection (including HIV, HCV and tuberculosis), addressing issues with pill burden and adherence. Better methods for scaling preclinical pharmacokinetics (PK) for prediction of human exposure are needed to aid decision making, and accelerate early clinical development through better human dose prediction.

Methods: Matching rat and human PK data were sourced from publications or in-house PK studies for 11 marketed intramuscular LAIs. Terminal depot release rates (KA) were determined from analysis of terminal phase, release-dependent, “flip-flop” half-lives. Two approaches for human KA prediction were: 1) Linear regression between human and rat KAs (Fig 1A). 2) Allometric scaling of KA by body size according to formula: KA (human, pred) = KA (rat) x (70 kg/0.3 kg) x 0.25 – KA (rat) x 0.255. Qualification was undertaken for cabotegravir and rilpivirine with human LAI PK profiles simulated for comparison with empirical human PK data. For this, human PK disposition was described by a minimal 1-compartment model parameterised with clearance (CL) and steady state volume of distribution (Vss) from published IV PK if available, or via PBPK where absent. This model was deemed sufficient under flip-flop PK due to slow LAI depot release masking multiphasic PK disposition. Depot release input was treated as a simplified 1st order process governed by KA. For qualification, the LAI of interest was removed from the regression used to predict the KA. Given that bioavailability (F) is unknown for novel LAIs and varies for approved LAIs, depot input was assumed 25, 50 and 100% F.

Results: A variety of PK profile shapes were evident across LAIs and terminal flip-flop KAs tended faster in rat than human. A good correlation was observed between human and rat KA with all 11 LAIs included (R2 = 0.81, slope coeff. 0.38), and retained with the removal of cabotegravir (R2 = 0.82) or rilpivirine (R2 = 0.81). Reasonable concordance of resulting human PK predictions was observed for cabotegravir (assuming 50% F; Fig 1A) and rilpivirine (assuming 100% F; Fig 1B).

Conclusion: This simplified scaling may be useful to predict human terminal release PK from rat studies to inform phase I human dose prediction. More work is needed to predict LAI F, and understand relationships across other species and routes of administration. Final validation of the approach will require a priori application for a novel LAI.

Pharmacokinetics of Long-Acting Cabotegravir and Rilpivirine in Elderly Using PBPK Modelling

Sara Bettonte, Mattis Beren, Felix Stader, Manuel Battegay, Catia Marzolini

University Hospital Basel, Basel, Switzerland, Certara, Sheffield, United Kingdom

Background: The quality of life of people with HIV (PWH) has significantly increased thanks to potent single pill antiretroviral regimens characterized by a good tolerability. Another milestone has been reached with long-acting (LA) formulations which enable infrequent dosing. Clinical trials of LA agents were mostly conducted in standard populations, leading to a lack of knowledge on their pharmacokinetics in elderly PWH. Physiologically based pharmacokinetic (PBPK) model is a mathematical tool approved by the regulatory authorities used to simulate clinical unknown scenarios. The aim of this study was to determine the pharmacokinetics of LA cabotegravir and rilpivirine in virtual elderly individuals.

Methods: Our in-house PBPK model built in Matlab® 2020a and implemented with an intramuscular framework was verified against clinical observed data for cabotegravir and rilpivirine after oral and intramuscular administration. As for PBPK modelling guidelines, the model was considered verified when the predictions were within 2-fold of clinical observed data. The effect of ageing on the pharmacokinetics of LA cabotegravir and rilpivirine was evaluated by using two separate cohorts of virtual individuals aged 20–50 (50% female) and 65–85 (50% female), respectively. The design of the ATLAS/FLAIR and ATLAS-2M studies was reproduced in our PBPK model and the fold change in elderly relative to young was determined for area under the concentration-time curve (AUC) and trough concentration (Cmin) at steady state.

Results: The PBPK model was successfully verified as all the predictions were within 2-fold of clinically observed data. Age related physiological changes did not significantly impact the Cmin and AUC of LA cabotegravir administered every month (Q4W), and the AUC of LA rilpivirine Q4W since the ratios (elderly vs young) were predicted to be within the bioequivalence range (0.8–1.25 fold). On the other hand, the Cmin of LA rilpivirine administered Q4W was 28% higher in elderly relative to young. Additionally, the Cmin and AUC of LA cabotegravir administered every other month (Q8W) were predicted to be 29% and 26% higher in elderly relative to young, respectively. Similarly, the Cmin and AUC of rilpivirine Q8W were 46% and 41% higher in elderly (Fig. 1).

Conclusion: Age related physiological changes are predicted to modestly increase the AUC and Cmin of LA cabotegravir and rilpivirine. Thus, elderly PWH could possibly be at lower risk for sub-optimal drug exposure at the end of the dosing interval.
Model-Based Comparison of Cabotegravir Pharmacokinetics Following Thigh and Gluteal Injections

Kelong Han, Ronald D’Amico, William Spreen, Susan Ford

GSK, Collegeville, PA, USA; Gilead Sciences, Inc, Foster City, CA, USA

Background: Cabotegravir (CAB) long-acting (LA) intramuscular (IM) gluteal injections are approved for HIV-1 pre-exposure prophylaxis (PrEP) and combination treatment with rilpivirine. The vastus lateralis (lateral) thigh muscle is a potential alternative site of administration in cases of gluteal injection fatigue or physical obstruction. We aimed to characterize CAB pharmacokinetics (PK) and its association with demographics following thigh administration in comparison to gluteal administration using population PK (PPK) analysis.

Methods: Fourteen participants (pts) who were HIV-negative and received a 600mg single thigh injection in Phase 1 Study 208832 and 118 pts who were HIV-positive and received thigh injections (400mg monthly (QM) x 4 or 600mg every-2-months (Q2M) x 2) after >3 years of gluteal injections in Phase 3b Study 207966 (ATLAS-2M) provided CAB concentrations for the analysis. An established gluteal PPK model was fit to PK data following both gluteal and thigh injections, enabling within-person comparison in ATLAS-2M pts. Gluteal parameters were fixed. Thigh parameters including absorption rate constant (KA-thigh) and bioavailability (F-thigh) were estimated. CAB PK profiles following chronic or intermittent thigh injections administered QM and Q2M were simulated and compared to gluteal injections. PK target was that 95% of pts maintain concentrations >0.45 µg/mL, the 5th percentile of observed concentrations from 366 thigh injections and 2022 concentrations from 1631 gluteal injections were analyzed. Similar to gluteal administration, KA-thigh was associated with sex and BMI. KA-thigh was correlated with and was generally faster than KA-gluteal, described by the additive linear relationship: KA-thigh = KA-gluteal + 0.000253 h⁻¹. Terminal half-life of thigh administration was 26% (male) and 39% (female) shorter than gluteal administration. F-thigh was 90% of gluteal injection. PK target was that 95% of pts maintain concentrations >0.45 µg/mL, the 5th percentile of observed CAB trough concentration following the gluteal initiation injection in Phase 3 Studies.

Results: 1254 concentrations from 366 thigh injections and 2022 concentrations from 1631 gluteal injections were analyzed. Similar to gluteal administration, KA-thigh was associated with sex and BMI. KA-thigh was correlated with and was generally faster than KA-gluteal, described by the additive linear relationship: KA-thigh = KA-gluteal + 0.000253 h⁻¹. Terminal half-life of thigh administration was 26% (male) and 39% (female) shorter than gluteal administration. F-thigh was 90% of gluteal injection. PK target was that 95% of pts maintain concentrations >0.45 µg/mL, the 5th percentile of observed CAB trough concentration following the gluteal initiation injection in Phase 3 Studies.

Conclusion: PPK modeling and simulation support chronic thigh administration of CAB LA QM and intermittent thigh injections for both QM and Q2M regimens. However, simulated chronic Q2M thigh administration did not maintain PK target established in pivotal trials and therefore is not recommended.

Effect of Broadly Neutralizing Antibody Exposure on HIV Rebound Following Combination Immunotherapy

Amelia N. Deitchman, Leonid Serebryannyy, Rowena Johnston, Lucio Gama, Marina Caixey, Elena Vendrame, Devi SenGupta, Romas Geleziunas, Jackie Reeves, Christos Petropoulos, Michel Nussenzweig, Rachel Rutlschauser, Steven G. Deeks, Michael J. Peluso

University of California San Francisco, San Francisco, CA, USA; National Institutes of Health, Bethesda, MD, USA; amfAR, New York, NY, USA; The Rockefeller University, New York, NY, USA; Gilead Sciences, Inc, Foster City, CA, USA; Monogram Biosciences, San Francisco, CA, USA

Background: Waning broadly neutralizing antibody (bNAb) levels and emergence of resistance have been associated with viral rebound during analytic treatment interruption (ATI) studies. In this pharmacokinetic/pharmacodynamic (PK/PD) analysis, we evaluated the impact of bNAbs exposure, susceptibility, and antidrug antibody (ADA) formation on rebound kinetics following combination immunotherapy with a boosted DNA vaccine, lefitolimod, and bNAbs (10-1074 and VRC07-523LS) (NCT04357821), in which 7/10 individuals exhibited altered post-intervention rebound dynamics.

Methods: We described plasma bNAbs PK using population PK modeling approaches in Monolix software. We performed Spearman correlations or Wilcoxon’s test to determine the relationship between bNAbs AUC, peak concentration (C₉₀), and bioavailability (F₉₀) level at time of rebound and rebound kinetics (time to rebound, post ATI setpoint). We evaluated susceptibility (IC₉₀) relative to bNAbs level (i.e., IQ₉₀; bNAb level/IC₉₀). We performed competitive and functional ADA assays for both bNAbs.

Results: Time to Rebound: Greater bNAb exposure for 10 participants (9 cis men, 1 trans woman) was associated with later rebound (VRC07-523LS AUC C₉₀ p=0.76; p=0.04; 10-1074 C₉₀ p=0.7; p=0.04). In those who rebounded later (>15 weeks), VRC07-523LS and 10-1074 were lower at the time of rebound (both p=0.01), with a similar trend observed for 10-1074 IC₉₀ (p=0.01 and p=0.03, for each bNAb). There was no association between IC₉₀ and time to rebound. Post intervention setpoints: Although there was no association between VRC07-523LS IC₉₀ and setpoint, higher VRC07-523LS IC₉₀ at the time of rebound was associated with higher post-intervention setpoint (p = 0.85; p=0.006). No association between 10-1074 IC₉₀ or IC₉₀ and setpoint were observed. Antidrug antibody: While ADA was detected for two participants for competitive assays for both bNAbs, no functional ADA impacting PK was observed.

Conclusion: Higher bNAb exposure (e.g., AUC, C₉₀) was consistently associated with delayed viral rebound. Our results suggest that post-treatment setpoint was not driven by bNAb susceptibility and that the association of IC₉₀ and setpoint is driven by higher VRC07-523LS levels at the time of rebound in those who rebounded earlier and had higher setpoints. Overall, bNAb PK/PD is likely not responsible for lower observed post-treatment setpoints during this trial, suggesting the effect is likely attributable to changes in anti-HIV immune function.

A Randomized, Adaptive Phase I Study to Determine the Phase II Dose of VIR-7832: AGILE CST5

Richard J. FitzGerald, Chris Edwards, Jimsan Periselneris, Geoff Saunders, Nicky Downs, Rebecca Lyon, Danny Pratt, Helen Reynolds, Lauren Walker, Gareth Griffiths, Saye Khoo, Thomas Fletcher, for AGILE CST

University of Liverpool, Liverpool, United Kingdom; NHRI Southampton Clinical Research Facility, Southampton, United Kingdom; King’s College Hospital NHS Foundation Trust, London, United Kingdom; University of Southampton, Southampton, United Kingdom; NIH Clinical Research Facility, Liverpool, United Kingdom; Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Background: Despite widespread COVID-19 vaccination, anti-virals remain important for COVID-19 treatment. Early treatment is crucial to avoid progression and severe disease, with monoclonal antibodies (mAb) offering possible advantages in single dosing and duration of effect. VIR-7832 is a novel mAb derived from the same parental antibody as sotrovimab with addition of an Fc domain XX2 modification to enhance effector function and modulate immune response. Here we report data from the First-in-Human (FIH) trial of VIR-7832 in mild-moderate COVID-19 patients (AGILE CST5, NCT04746183).

Methods: Participants with PCR-confirmed mild-moderate COVID-19 infection, within 7 days of onset, were randomised (3:1, double-blind) to single dose VIR-7832 or placebo. Three cohorts of 8 participants were recruited, with dose escalation in between (Cohort 1-50mg, Cohort 2-150mg, Cohort 3-500mg) supported by a Bayesian dose-toxicity model. Participants were followed to day 253 post-dose, with assessments performed for safety (laboratory samples.
Gendered Inclusion in Studies Evaluating Injectable HIV Treatment and PrEP: A Scoping Review

Alexa L. Elias, Isabelle Whelan, Melanie Smuk, Nuala A. Pepper, Nishat Halim, Vanessa Apea, Chloe M. Orkin

Queen Mary University of London, London, United Kingdom

Background: Globally, 54% of people living with HIV (PLWH) are female (UNAIDS). Therapies containing long-acting injectable (LA-I) agents are included in treatment guidelines and licensed for pre-exposure prophylaxis (PrEP). In female participants, LA-I cabotegravir (CAB) is superior to oral PrEP. However, cisgender and transgender women are historically underrepresented in wider HIV research.

Methods: We report an ongoing scoping review to evaluate inclusion of women (cis/trans) and non-binary people (NNBP) in Phase I-III clinical trials, implementation and real-world studies evaluating any LA-I agent for HIV treatment or PrEP. We searched electronic databases (PubMed, MEDLINE, Embase) and peer reviewed abstracts excluding studies with no report of sex or gender. Statistics were descriptive.

Results: We included 41 studies, 14 evaluated PrEP (34%) and 27 (66%) evaluated treatment. We included 27 clinical trials (Phase I (n=8); Phase II (n=12); Phase III (n=7)), 6 implementation and 8 real-world studies. 38 studies included sites in high-income countries and 13 included sites in low-and middle-income countries. PrEP studies evaluated CAB 8 (57%), rilpivirine (RPV) 2 (14%) and mono- or clonal antibodies 4 (29%). Treatment studies evaluated CAB+RPV, lenacapavir, abacavir and monoclonal antibodies, 18 (67%), 5 (18%), 3 (11%), 1 (4%), respectively. The number of female participants increased 98-fold in PrEP studies (2014-present), and 361-fold in treatment studies (2010-present). However, the percentage of female participants in LA-I treatment studies during 2022/3 remains disappointing [724/3709 (19%)].

Conclusion: This is the first report of the safety, tolerability and viral outcomes for VIR-7832, a novel mAb for treating SARS-CoV-2. These data suggest VIR-7832 is safe and well tolerated at doses up to 500mg. SARS-CoV-2 PCR data are reported (dyspnoea, considered unrelated) was reported in a participant receiving 50mg VIR-7832. Skin rashes were reported by 2 participants receiving 150mg VIR-7832 (lichenoid reaction & maculopapular rash). Time to negative SARS-CoV-2 PCR in all participants in each cohort was 15 days for those receiving 50mg VIR-7832, 8 days for 150mg VIR-7832, 8 days for 500mg VIR-7832 and 22 days for placebo.

621 Implementing LA Cabotegravir (CAB) + Rilpivirine (RPV) Therapy in 6 UK Clinics & in the Community: ILANA

Chloe M. Orkin, Joanne S. Haviland, Yuk L. Wong, Sara Paparini, Bakita Kasadha, Rosalie Hayes, Julie Fox, Ruth Byrne, Amanda Clarke, Emily Clarke, Tristan J. Barber, Vanessa Apea, for the ILANA Study Group

1 Queen Mary University of London, London, United Kingdom, 2 University of Oxford, Oxford, United Kingdom, 3 King’s College London, London, United Kingdom, 4 Chelsea and Westminster NHS Foundation Trust, London, United Kingdom, 5 Brighton and Sussex University Hospitals NHS Trust, Brighton, United Kingdom, 6 Royal Liverpool University Hospital, Liverpool, United Kingdom, 7 Royal Free Hospital, London, United Kingdom

Background: The feasibility of implementing CAB+RPV LA ART in the community has not yet been described. In the first UK-based study, we purposefully recruited participants more representative of the global population of PLWH to evaluate feasibility of implementation in 6 clinics (in & outside London) and in the community.

Methods: ILANA is a 1-year UK-based implementation study in PLWH who switched to CAB+RPV LA 2-monthly within the label. Participants receive CAB+RPV in the clinic for 6 months with an option to receive the drug in the clinic or community (eg home or community organization) from M6-M12. This prespecified M4 analysis evaluated PLWH perspectives on feasibility and acceptability of CAB+RPV LA and on potential community delivery through validated implementation questionnaires (Feasibility of Intervention Measure (FIM), Acceptability of Intervention Measure (AIM), Intervention Appropriateness Measure (IAM), HIV Treatment Satisfaction Questionnaire (HIVTSQ-12)) at baseline and 4 months.

Results: Between May-December 2022 we enrolled 114 virally suppressed PLWH [53% female, 51% Black, 30% White, 40%> 50yrs, 68% heterosexual,75% employed]. Median time since diagnosis was 13 yrs (IQR 8, 19), time on ART was 11 yrs (IQR 7.16) with a mean of 3 prior regimens. 57% had received NNRTIs. 84% had other co-morbidities, and 80% (91/114) were taking non-AIDS medications. 75% (68/91) were on >2 non-AIDS drugs. 99% of injections were given within the 7-day window. There were 5 discontinuations by M4 (2 injection-related, 3 participant choice). 1 participant with VL 478 at M4 continued. Regarding injectable treatment: FIM, IAM and HIVTSQ scores improved significantly (baseline to M4) (Table). FIM, IAM and AIM scores regarding perceptions of the future prospect of receiving ART in the community setting (at M6) did not improve from baseline. 96% preferred injectable to oral ART at M4. At M4, IAM mean scores were significantly less favourable in Black participants than in White participants with respect to the injection (4.44 vs. 4.67, p=0.029) and the community setting (3 vs 3.75;p=0.003).

Conclusion: Over four months, participants found injections increasingly feasible, appropriate and satisfactory, and preferred them to oral ART. Pre-specified sub-group analyses based on gender and race and ethnicity revealed significant differences around perception of delivering LA ART in the community in Black participants. These findings will be further evaluated in relation to psychological challenges.
622 Acceptability of Long-Acting Cabotegravir & Rilpivirine in a Large Urban Amphibolic HIV Clinic

Casey M. Luc, Blake Mae, Sara Perez, Kara Herrera, Mark Dworkin
University of Illinois at Chicago, Chicago, IL, USA, Public Health Care Center of Chicago, IL, USA

Background: There are limited data regarding the acceptability of injectable long-acting cabotegravir and rilpivirine (LA-CAB/RPV) outside of randomized controlled trials (RCTs). We performed a mixed-methods analysis to describe patient-reported outcomes (PROs) of LA-CAB/RPV among a population often underrepresented in RCTs at one of the largest HIV/AIDS care centers in the United States.

Methods: We interviewed persons living with HIV (PLWH) who received at least one dose of LA-CAB/RPV at the Ruth M. Rothstein CORE Center in Chicago, Illinois. PRO endpoints included mean treatment satisfaction (1 [Very Unsatisfied] to 7 [Very Satisfied]), mean tolerability of injection site pain (1 [Not at All Bothered] to 10 [Very Bothered]), and reasons for switching to LA-CAB/RPV. Mean and standard deviations (mean±SD) and proportions (%) are reported.

Results: Among the respondents (N=136), 68.4% identified as Black/African American, 24.3% as Hispanic, 36.0% as female, 58.1% as male; the median age was 43 years (range 21-76), with 36.8% being ≥50 years old. Most respondents (92.6%) completed ≥3 injection appointments at the time of interview. The two most common reasons for switching from oral therapy to LA-CAB/RPV were no longer wanting to take pills (89.7%) and had trouble taking their pills daily (85.5%). Treatment satisfaction was high (6.7±0.4). Two-thirds (64.0%) reported an aspect of their life improved that was not expected after initiation. The majority (90.4%) reported pain from injections, with a mean pain level of 4.2±2.7. Among those reporting pain, half (48.8%) reported pain decreased after initial injection. Among those with a reported >6 level of pain (N=31), most (83.9%) reported no improvement since initial injection. One-fifth (19.1%) reported swelling from injections and one-third (33.1%) reported anxiety before injections.

Conclusion: We found high treatment satisfaction with LA-CAB/RPV in a population with a high proportion of people of color, women, and PLWH ≥50 years old. Patients reported moderate pain with injections, which improved with time. These results suggest that scaling up of injectable LA-CAB/RPV will be met with high patient acceptability across diverse patient populations.

623 Real-World Effectiveness of Cabotegravir + Rilpivirine vs Standard of Care Oral Regimens in the US

Ricky K. Hsu1, Michael Senzon2, Jennifer S. Fusco2, Laurence Brunet1, Quateeka Cochran3, Gayathri Sridhar1, Vani Vannappagari1, Jean Van Wyk2, Michael B. Wohlfeiler2, Brooke Levis3, Gregory P. Fusco2
1AIDS Healthcare Foundation, New York, NY, USA, 2C4N Community Health, Sarasota, FL, USA, 3University of California San Francisco, San Francisco, CA, USA

Background: In trials, long-acting (LA) injectable antiretroviral therapy (ART) with cabotegravir plus rilpivirine (CAB+RPV) was shown to be non-inferior to oral ART regimens in virologically suppressed (viral load [VL] <50 copies/mL) individuals. We assessed real-world effectiveness after a switch to CAB+RPV or a new oral ART regimen.

Methods: From the OPERA cohort, ART-experienced, suppressed adults with HIV switching to CAB+RPV or a new oral ART regimen between 21Jan2021-31Dec2022 were followed through 30June2023. Confirmed virologic failure (VF; 2 VL ≥200 copies/mL or 1 VL ≥200 copies/mL + regimen change) was assessed among those with ≥1 follow-up VL. Logistic regression models were fit to assess the risk of VF by regimen, adjusted for age (linear & quadratic terms), sex, race, injection drug use (IDU), history of AIDS-defining events (ADE), CD4 count (linear & quadratic terms), comorbid conditions, and prior regimen class. In those receiving CAB+RPV injections, age, sex, race, US region, IDU, history of ADE, CD4 count (per 100 cells/μL), comorbid conditions, prior regimen class, and BMI were evaluated as potential predictors of VF.

Results: In OPERA, 1362 virologically suppressed adults switched to CAB+RPV injections and 2783 switched to a new oral ART regimen. CAB+RPV users were younger (aged ≥50 years: 29% vs. 41%), had been on their prior regimen for a shorter period (20 vs. 37 months), were more likely to switch from an INSTI (74% vs. 68%), but had similar median CD4 counts at initiation (668 [IQR 496-902] vs. 700 [524-913] cells/μL) compared to oral ART users. Risk of VF out of individuals with a follow-up VL (CAB+RPV: n=1236; oral ART: n=2492) did not statistically differ (adjusted OR: 0.64; 95% CI: 0.41, 1.02). Only baseline CD4 marginally predicted VF, every 100 CD4 cells/μL increase was associated with 15% lower risk of VF (Fig 1). Of the 25 CAB+RPV VF, 40% went to INSTI oral therapy, 40% remained on CAB+RPV, 16% went to multi-core agents, and 1 remained off therapy. Of the 19 with VL, 95% achieved <200 and 79% <50 after VF. In contrast, of the 78 oral VF, 69% stayed on the same ART, 23% went to INSTI regimen, 3 remained off therapy, and the remainder went on a variety of other regimens. The 43 with VL, 84% achieved <200 and 72% <50 after VF.

Conclusion: In routine clinical care in the US, the risk of VF did not differ between virologically suppressed adults switching to CAB+RPV injections or oral ART regimens. Lower CD4 count at initiation was the only predictor of VF for CAB+RPV.

624 Predictors of Injection Visit Adherence in Those Receiving Injectable Cabotegravir/Rilpivirine

Lucas Hill, Jeffrey Yin, Nimish Patel, Kari Abulhosn, Elvia Suarez, Afsana Karim, Laura Bambard
University of California San Diego, La Jolla, CA, USA

Background: Long-acting injectable (LAI) cabotegravir/rilpivirine (CAB/RPV) provides a novel treatment option for people with HIV (PWH). However, missed and late injections potentially jeopardize viral suppression, increases risk of resistance development, and requires extensive resources to track adherence and proactively reschedule missed injections.

Methods: We conducted a retrospective cohort study at the University of California San Diego HIV Clinic. Adult PWH who received LAI CAB/RPV for at least 6 months from May 2021 through August 2023 were included. Data collected included demographics, baseline HIV RNA and CD4 count, distance from the clinic, substance use, CAB/RPV dosing regimen, office visit no-shows one year prior to switching to LAI CAB/RPV, injection visit no-shows, injections
Results: A total of 287 PWI were included with median age of 42 years, 54.4% were non-white, 38% Hispanic ethnicity, and 15% female sex assigned at birth (SAB). Median follow-up time (IQR) on LAI CAB/ RPV was 450 days (344–548 days), and median distance to the clinic was 4.8 miles (2.5–11.8 miles). A total of 92 (32.1%) had at least one no-show to a scheduled injection visit and 44 (15.3%) had at least one injection outside the recommended dosing window. Younger age (HR 0.97, 95%CI 0.95–0.98) and ≥ 1 office visit no-show in the year prior to switch (HR 2.03, 95%CI 1.32–3.12) were independently associated with having a no-show to an injection visit (Figure 1). The number of pre-switch no-shows was also significantly associated with post-switch no-shows (p<0.001) and those with at least one injection visit no-show had a higher frequency of late injections, 30.4% vs. 8.2%, p<0.0001. Male SAB (HR 9.18, 95%CI 1.26–66.9) after adjustment for age, was independently associated with late injection visits. There was no relationship between no-shows to injection visits or late injections and having a detectable viral load or virologic failure (n=3) after switch to LAL CAB/RPV.

Conclusion: Evaluating attendance to office visits prior to switching to LAL CAB/RPV may help identify those more likely to miss injection visits, however it was not associated with late injections or having a detectable viral load or virologic failure.

Table: Characteristics of ART-experienced, virologically suppressed PWI with LA injection

| Study Population Characteristics | All CAB-RPV Initiators | N (%)
|---------------------------------|-----------------------|-------
| Age, median (IQR) | 44 (25, 55) |
| Male sex, n (%) | 223 (79.5) |
| Race, n (%) | 35 (12.9) |
| Geographic region, South, n (%) | 204 (73.4) |
| On CAB-RPV regimen at end of follow-up, n (%) | 250 (88.5) |
| CAB+RPV initiators with ≥ 2 recorded follow-up visits, n (%) | 221 (79.5) |
| Maintained virologic suppression, n (%) | 233 (96.6) |
| Confirmed virologic failure, n (%) | 2 (0.9) |

626 Real-World Virologic Outcomes of Cabotegravir/Rilpivirine in Patients With Elevated Body Mass Index

Christina Maguire 6, Eric Farmer 4, Emily Huesgens, Kaitlyn Rueve 5, Marisa Brizzi 2, Amanda Binkley 4, Bernice Kear 4, Pallavi Chary 5, Helen Koenig 5, Peter Sung 5, Emily Hiserott 5, Karam Mounzer 5, Danielle Rocha 6, Adrian Alterm 5, William R. Short 6, University of Pennsylvania, Philadelphia, PA, USA; Indiana University, Bloomington, IN, USA; University of Cincinnati, Cincinnati, OH, USA; Drexel University, Philadelphia, PA, USA; Philadelphia FIGHT, Philadelphia, PA, USA; Philadelphia FIGHT, Philadelphia, PA, USA

Background: Injectable cabotegravir (CAB) and rilpivirine (RPV) is the first complete long-acting antiretroviral (ARV) regimen approved in persons with HIV with sustained virologic suppression (HIV RNA <50 copies/mL) on current ARV therapy. In a multivariable analysis, body mass index (BMI) >30 kg/m² alone was not predictive of confirmed virologic failure; however, data are limited due to small sample sizes. The primary objective of this study is to determine the incidence proportion of participants with a plasma HIV-1 RNA >50 copies/mL at last observed endpoint in those with and without body mass index >30 kg/m². Secondary objectives evaluated include incidence proportion of virologic failure (VF: >200 HIV-1 RNA copies/mL) in those with and without body mass index >30 kg/m² and assessing the primary outcome stratified by BMI categories (>40 kg/m², >45 kg/m², and >50 kg/m²).

Methods: We conducted a retrospective, multi-center cohort study from January 22, 2021 to February 15, 2023 for participants receiving either every 4 or 8 weeks dosing of CAB/RPV (Q4w or Q8w). We included participants who were >18 years of age, received at least one injection of CAB/RPV, had HIV RNA <50 copies/mL at baseline, and had at least one follow up HIV RNA. We excluded those with no weight available within 90 days of first CAB/RPV injection. Baseline characteristics such as prior regimens, resistance history, smoking history, and history of gluteal implants were collected. HIV RNA >50 copies/mL and virologic failure (VF: >200 HIV-1 RNA copies/mL) were collected. Data was pooled to determine the proportion of participants with HIV RNA >50 copies/mL in those with BMI >30 kg/m² compared to those who with BMI <30 kg/m².

Results: We analyzed 369 participants across 5 medical centers and 148 (40.1%) had a BMI >30 kg/m². Individuals received a median 202 days of therapy (range 22-664). Sixteen (4.3%) were on Q4w, 250 (67.8%) were on Q8w, and 103 (27.9%) were on a combination Q4-Q8w. For those with BMI >30 the median BMI was 35.1 (range 30.0-67.4) kg/m². Eighteen individuals (4.8%) had HIV RNA >50 copies/mL with BMI >30 kg/m² compared to 20 (5.4%) with a BMI <30 kg/m². Additionally, we found that 3 individuals (0.8%) experienced VF at the last timepoint with BMI >30 kg/m² compared to 4 (1.04%) with a BMI <30 kg/m².

Conclusion: Based on our analysis, the incidence of HIV RNA >50 copies/mL and VF were similar between groups regardless of BMI category.
627  HIV-1 RNA Blips and Low-Level Viral Replication: SOLAR (CAB+RPV LA vs BIC/FTC/TAF)

Christine Latham1, Rimgaila Urbaitiene1, Kenneth Sutton1, William Spreen1, Ronald D’Amico1
1ViiV Healthcare, Durham, NC, 1GlaucSmuthHline, Brentford, United Kingdom

Background: Cabotegravir plus rilpivirine long-acting (CAB+RPV LA) administered every 2 months (Q2M) is the first and only complete LA regimen recommended for virologically suppressed people living with HIV-1. Here, we report HIV-1 RNA viral blips and target virus not detected (TND), as well as the impact of HIV-1 RNA blips on viral load measurements at Month 12 and confirmed virologic failure (CVF), in participants switching to CAB+RPV LA vs. continuing daily oral cabotegravir/emitricabintab/rilpivirine alafenamide (BIC/FTC/TAF) through Month 12 in the SOLAR study.

Methods: SOLAR (NCT04542070) is a Phase 3b, randomized (2:1), open-label, multicenter, noninferiority study assessing virologically suppressed adults to CAB+RPV LA Q2M vs. continuing BIC/FTC/TAF. The analysis was based on the modified intention-to-treat exposed (mitIT-E) population (exclusion of one trial site for non-compliance to protocol entry criteria). HIV-1 RNA viral blips were defined as a single HIV-1 RNA value between 50 and <200 c/mL with adjacent values <50 c/mL. CVF was defined as two consecutive HIV-1 RNA ≥200 c/mL values. Plasma samples were analyzed for HIV-1 RNA viral load using the Abbott RealTime HIV-1 assay, and TND outcomes were provided for HIV-1 RNA <40 c/mL.

Results: Of 670 participants (mitIT-E), 447 (67%) switched to LA and 223 (33%) continued BIC/FTC/TAF. The proportion of participants with HIV-1 viral blips through Month 12 was 4% (n=19/447) in the CAB+RPV LA arm and 4% (n=9/223) in the BIC/FTC/TAF arm. Of participants with viral blips, 5% (n=1/19) and 11% (n=1/9) in the CAB+RPV LA and BIC/FTC/TAF arms, respectively, had HIV-1 RNA ≥50 c/mL at Month 12; no participants with HIV-1 RNA viral blips developed CVF. The proportions of participants with viral blips were consistently ≤1% of participants with available data across both treatment arms at any time point. TND outcomes at individual study visits were similar between study arms (CAB+RPV LA, 85–88%; BIC/FTC/TAF, 80–86%), and the proportions of participants with HIV-1 RNA <40 c/mL (CAB+RPV LA, 90–97%; BIC/FTC/TAF, 90–97%) were comparable between treatment arms through Month 12.

Conclusion: The proportions of study participants with HIV-1 RNA viral blips, TND, and HIV-1 RNA <40 c/mL were similar between CAB+RPV LA and BIC/FTC/TAF through Month 12. HIV-1 viral blips with CAB+RPV LA did not appear to be associated with CVF, consistent with prior CAB+RPV LA Phase 3 clinical study data.

Case Series Examining the Long-Acting Combination of Lenacapavir and Cabotegravir: Call for a Trial

Monica Gandhi1, Lucas Hill2, Janet Grochowski1, Alexander Nelson1, Katerina Christopoulos1, Diane Havlir1, Catherine A. Koss1, Jillian Baron1, John D. Szumowski1, Jon Oskarsson1, Mary Shiels1, Samantha Dilworth, Ayesha Matt1, John D. Szumowski1, Ann Avery2, Laura Bamber2, William R. Short3, Corrilyn O. Hileman4
1University of California San Francisco, San Francisco, CA, USA, 2University of California San Diego, La Jolla, CA, USA, 3MetroHealth Medical Center, Cleveland, OH, USA, 4University of Pennsylvania, Philadelphia, PA, USA

Background: Long-acting antiretroviral therapy (LA-ART) is novel and has been used for both virologically-suppressed (VS) and viremic patients with adherence challenges. The currently-approved LA ART combination — cabotegravir (CAB) and rilpivirine (RPV) — is limited by the relatively high prevalence (up to 10%) of nonnucleoside reverse transcriptase inhibitor (NNRTI) resistant virus globally and is not endorsed for low-and-middle-income-countries (LMICs). The combination of LA lenacapavir (LEN) and CAB has not been studied in a clinical trial but poses promise for LMICs.

Methods: Providers (MDs/pharmacists) at the UCSF Ward 86 Clinic, the UCSF Owen Clinic, MetroHealth in Cleveland, and the UPenn HIV Clinic used LEN subcutaneously every 6 months after oral loading in combination with CAB administered intramuscularly every 4 or 8 weeks in patients with adherence challenges to oral ART. Close follow-up was conducted after the outcome window (sensitivity analysis). We also describe viral failure (VF), defined as <2-log viral load decline at 4 weeks or VL ≥200 copies/ml at initial VS with emergent CAB- or RPV-associated resistance mutations; and overall VS at week 24 including those who switched to oral ART.

Results: Among 243 PWI initiating LA-CAB/RPV, 88 (36%) had baseline VL ≥50 copies/mL and 60 had ≥32 weeks of follow-up time to assess the primary outcome (88% cisgender men, 47% age ≥50, 40% white, 28% Latino/a, 25% Black, 47% with housing instability, 43% using stimulants). At 24 weeks, 51 (85%) had VS, 4 had VL ≥50 copies/mL and 5 had missing VL data (table).

40-nine met the primary outcome of LA-CAB/RPV persistence and VS (82%; 95%CI 69–90%), with this estimate rising to 85% (52/60) using imputed VLs in sensitivity analysis. Of the four with VL ≥50 copies/mL at week 24, two had VF with resistance (RT E138K, INSTI R263K, RT L100I, Y181I) and two had slow viral decay. Without week 24, two patients with VF later attained VS on alternative regimens (lenacapavir/LEN+BIC/TAF/TAF and CAB+LEN). Four other patients discontinued LA-CAB/RPV before week 24: two had VS on oral ART at week 24, one had VS after switch to oral ART but missing week 24 VL, and one was off ART and lost to follow-up due to psychosis. Week 24 VS on either LA-CAB/RPV or oral ART using imputed VL data was 92% (55/60).

Conclusion: In those initiating LA-CAB/RPV without viral suppression, 24-week VS estimates were at least 85%. Long-acting ART can be an important tool for improving VS among patients who face adherence challenges to oral ART.

628 24-Week Viral Suppression in Patients Starting Long-Acting CAB+RPV Without HIV Viral Suppression

Matt Hickey, Janet Grochowski, Francis Mayorga-Munoz, Elizabeth Imbert, John D. Szumowski, Jon Oskarsson, Mary Shiels, Samantha Dílworth, Ayesha Appa, Diane Havlir, Monica Gandhi, Katerina Christopoulos
University of California San Francisco, San Francisco, CA, USA

Background: We previously demonstrated that initiation of long-acting cabotegravir/rilpivirine (LA-CAB/RPV) in people with HIV (PWH) with an unsuppressed HIV viral load (VL) at the Ward 86 clinic in San Francisco can rapidly lead to viral suppression (VS). We now seek to evaluate the durability of VS in this population.

Methods: We conducted a retrospective cohort study of PWH who started LA-CAB/RPV before 7/17/2023, focusing on those with HIV VL ≥50 copies/mL at initiation. Our primary outcome was VS (VL <50 copies/mL) and LA-CAB/RPV persistence (not discontinued or late by >14 days) at 24 weeks, using the closest VL to 24 +/-8 weeks. We considered missing 24-week VL as 1) unsuppressed (primary analysis); and 2) suppressed if evidence of VS before and after the outcome window (sensitivity analysis). We also describe viral failure (VF), defined as <2-log viral load decline at 4 weeks or VL ≥200 copies/ml after initial VS with emergent CAB- or RPV-associated resistance mutations; and overall VS at week 24 including those who switched to oral ART.

Results: Of 447 participants (mitIT-E), 172 (38%) switched to LA and 275 (62%) continued BIC/FTC/TAF. The proportions of participants with viral blips, TND, and HIV-1 RNA <40 c/mL were similar between LA-CAB/RPV and BIC/FTC/TAF through Month 12. HIV-1 viral blips with LA-CAB/RPV did not appear to be associated with VF, consistent with prior CAB+RPV LA Phase 3 clinical study data.
Lenacapivir Efficacy in CAPELLA Patients With No Fully Active Agents
In Optimized Background Regimen

Onyema Ogbugho1, Winali Ratanaasawat1, Anchalee Arvingsan2, Poenchan Chetchotisakdi1, Andrew Wiznia1, Kimberly Workowski1, Chien-Ching Hung1, Jason Brunetta1, Benoit Trottier1, Mhammed Rassoul1, Hui Wang1, Nicolas Margott1, Hadas Dvory-Sobol1, Martin S. Rhees1, Sorana Segal-Maurer2
1Iuide University, New Haven, CT, USA, 2Mahidol University, Bangkok, Thailand, 3Tha Red Cross AIDS Research Center, Bangkok, Thailand, 4Khon Kaen University, Khon Kaen, Thailand, 5Albert Einstein College of Medicine, Bronx, NY, USA, 6Emory University, Atlanta, GA, USA, 7National Taiwan University Hospital, Taipei, Taiwan, 8Maple Leaf Medical Clinic, Toronto, Canada, 9Clinique Médicale du Quartier Latin, Montreal, Canada, 10University of the Witwatersrand, Johannesburg, South Africa, 11Colden Sciences, Inc, Foster City, CA, USA, 12New York Presbyterian Hospital, New York, NY, USA

**Background:** Lenacapivir (LEN), a long-acting HIV-1 capsid inhibitor, is approved for the treatment of heavily treatment-experienced (HTE) people with HIV (PWH) with multidrug resistance (MDR) in combination with other antiretrovirals (ARVs). LEN is highly potent with no overlapping resistance with other ARVs. In CAPELLA, LEN in combination with an optimized background regimen (OBR) led to high virologic suppression: 78% (n=56/72; week [W] 52). We assessed LEN efficacy in participants whose OBR had no fully active ARVs.

**Methods:** The Phase 2/3 CAPELLA study enrolled HTF PWH with MDR. Eligible participants had resistance to ≥2 ARVs in ≥3 of the 4 main ARV classes (NRTI, NNRTI, PI, INSTI). After oral loading, SC LEN was administered every 6 months. OBRs were selected by the clinicians; other investigational drugs were permitted. OBR overall susceptibility score was the sum of susceptibility scores for each OBR ARV; 0 (no susceptibility), 0.5 (partial susceptibility) and 1 (full susceptibility). Efficacy data (HIV-1 RNA copies/mL; FDA Snapshot algorithm) were assessed at W26, 52, and 104. LEN and OBR ARV resistance analyses were done at virologic failure (virologic rebound ≥50 copies/mL or <1 log10 decline vs baseline).

**Results:** Of the enrolled 72 participants, 12 (17%) had no fully active ARVs in their OBR; 6/12 and 1/12 had 1 or partially active (score 0.5 each) ARVs, respectively. Median (range) number of OBR ARVs was 4 (2–6). Overall, OBR comprised NRTI (9 participants), INSTI (8), PI (7) or NNRTI (6); 5, 2, and 2 participants were on a CD4 post-attachment inhibitor (ibalizumab), CCR5 inhibitor (maraviroc), or attachment inhibitor (fostemsavir), respectively. Treatment outcomes: 8 participants had HIV-1 RNA <50 copies/mL at all 3 visits (W26, 52, and 104), including 1 participant with LEN resistance (R; M66I) at W10 and an OBR change at W25. 1 participant with missing W104 data was suppressed at a later visit; 1 participant not suppressed at W26 developed LEN-R (M66I) but was suppressed at W52 and 104 (OBR changed at W25); and 2 participants had HIV-1 RNA ≥50 copies/mL throughout, but with a stable, low-level viral load (1 with <600 copies/mL; OBR changed at W30; 1 with <3000 copies/mL despite emerging LEN-R at W4 [M66I]). None of the 12 participants discontinued the study drug.

**Conclusion:** In HTF PWH with MDR on an OBR with no fully active ARVs, LEN led to sustained virologic suppression over 104 weeks for most participants. LEN is an important option for treating HTF PWH with MDR.

631 Intramuscular Injection vs Intravenous Infusion of Ibalizumab for HTF PWH: The Results of TMB-302

Kaitlin R. Anstett1, R. Brandon Cash4, Anthony Mills3, Megezebe Berhe1, Edwin Delesalle3
1Theratechnologies, Inc, Montreux, Canada, 2Men’s Health Foundation, Los Angeles, CA, USA, 3 Baylor Institute for Immunology Research, Dallas, TX, USA, 4Orlando Immunology Centre, Orlando, FL, USA

**Background:** Ibalizumab (IBA) is a monoclonal antibody approved for the treatment of multi-drug resistant HIV-1 infection in heavily treatment experienced (HTE) people with HIV (PWH) in combination with an optimized background regimen. The efficacy of ibalizumab (IBA) in combination with an optimized background regimen has been demonstrated in clinical trials previously. IBA is currently administered every two weeks (Q2W) via intravenous infusion (IVI) over 15-30 minutes, or via undiluted intravenous push over 30 seconds. This requires a peripheral IV catheter for administration by trained staff, which can limit access. Altering the mechanism of administration of IBA will expand and simply access for HTF PWH in need of new therapies to achieve their treatment goals.

**Methods:** TMB-302 (ClinicalTrials.gov Identifier: NCT03913195) is a phase 3 study of the safety and efficacy of IBA administered as an intramuscular (IM) injection in clinically stable HIV-1 infected IBA experienced patients and healthy HIV-uninfected volunteers. All subjects in this study received at least two IVI infusions of IBA prior to administration via IMI injection for 8 weeks. The HIV treatment satisfaction questionnaire – status (HIVTSQS) and study medication satisfaction questionnaire - status (SMQs) were administered at the last IVI administration (day 29) and after 8 weeks of IM administration (day 85) and the HIV treatment satisfaction questionnaire – change (HIVTSQC) and study medication questionnaire - change (SMQC) were also administered on day 85. All study participants were also asked to answer a one-question Preference Assessment at Day 85 comparing the experience on IM injections with IV infusion.

**Results:** Administration of IBA via both IM and IV were well-tolerated by study participants. Although there was no statistically significant difference in either the HIVTSQS or SMQs between day 29 and 85, 83% of HIV-uninfected volunteers and 67% of HIV-infected clinical stable participants preferred administration of IBA IM compared to IVI. Importantly, no PWH lost viral suppression throughout the course of the study. No new or unexpected safety signals were identified.

**Conclusion:** Administration of IBA via IM injection was safe, well-tolerated, did not result in the loss of viral suppression among HTF PWH, and was the preferred method of administration of all participants in the TMB-302 study. Expanding the administration options for IBA is an important step to increasing access and agency for PWH.
633 The Preclinical Profile of Maturation Inhibitor VH3739937
Brian McAlliffe, Paul Falk, Ira Dicker, Susan Jenkins, Jean Simmermacher, Mark Krystal
ViiV Healthcare, Branford, CT, USA

Background: VH3739937 (VH37) is a next-generation maturation inhibitor (MI) currently in clinical trials. We report here on its preclinical profile.

Methods: Replication competent and single cycle inhibition assays were performed using a cohort of clinical isolates or site-directed mutant (SDM) viruses. Biochemical experiments using purified viral like particles (VLPs) were performed, while a radiolabeled surrogate compound was used to examine binding kinetics to viral-like particles (VLPs). Resistance selection was carried out using N14-3 virus at escalating concentrations of VH37. DMPK studies were also carried out.

Results: Biochemical experiments showed VH37 to be a bona fide MI. VH37 is a pan-genotypic MI that was active against all HIV-1 virus examined. EC50 of 1-5 nM were obtained against 8 laboratory strains and 43 clinical isolates. VH37 was also highly potent against all SDM polymorphisms previously identified as causing reduced susceptibility to first generation MIs. VH37 was partially active against a replication competent A36V4 mutant, with an EC50 of 5 nM and a maximum percent inhibition (MPI) of 95%. However, A36V4 could be selected under escalating VH37 conditions in cell culture, potentially explained by its weaker activity in a single cycle inhibition assay (EC50=32 nM; MPI=57%). Other mutations selected by VH37 included T332P, L363W or the triple mutant H14V/V362I/R384K. However, whereas each of these mutations induced highly reduced susceptibility in a single cycle assay, incorporation of any of these mutations into a replicating virus clone did not produce functional virus. The surrogate compound bound to an NLP VLP with a binding affinity of 3.3 ± 0.8 nM and had a dissociative half-life of 4125 minutes (almost 3 days), showing the compound binds avidly and dissociates slowly. This was in agreement with SPI cleavage data obtained with VLPs. Finally, VH37 was -93.3% protein bound in 100% human serum and exhibited DMPK profiles suitable for clinical development.

Conclusion: The pre-clinical virology profile of VH37 supported the progression of this MI into clinical development.

634 Next-Generation Maturation Inhibitor GS3K640254 Showed Broad Spectrum Potency Without MI Resistance
Jerry L. Jeffrey, Tom White, Samit Joshi, Brian Wynne
ViiV Healthcare, Research Triangle Park, NC, USA, ViiV Healthcare, Branford, United Kingdom

Background: HIV-1 maturation inhibitors (MI) offer a novel mechanism of action but have suffered from gag polymorphisms and decreased antiviral potency. GS3K640254 (GSK'254) is a next-generation MI that demonstrated broad spectrum coverage of gag polymorphisms in vitro (Dicker et al. AAC 2022;66:e0187621). GSK'254 + 2 nucleoside-reverse transcriptase inhibitors (NRTIs) demonstrated comparable efficacy when compared to DTG + 2 NRTIs with no treatment-emergent resistance against all assayed viruses. GS3K640254 is a potent and selective inhibitor of HIV-1 replication in the DOMINO Phase 2b dose-ranging study (Joshi et al. EACS 2023. Oral). In vitro analysis of GSK'254 predicted activity against a broad array of gag polymorphisms, including those hindering prior MI class compounds. Clinical data from the DOMINO study supported the efficacy of this novel MI, GSK'254, as no virus tested from the study demonstrated a decrease in potency to GSK'254 at baseline, through week 4 on treatment, or at any time of protocol defined virologic failure. The clinical durability observed for GSK'254 in this study suggests a sufficient genetic barrier without emergence of resistance.

Overall, these data provide clinical evidence that newer MI class compounds offer future options for HIV-1 treatment without the issue of gag polymorphisms observed with prior MI compounds. Finally, the learnings from this Phase 2b study will support the development of future MI compounds in the VIIV pipeline.

635 Structure-Guided Optimization of InSTIs to Increase Antiviral Potencies Against HIV-1 IN Mutants
Steven Smith, Xue Zhi Zhao, Stephen Hughes, Terrence R. Burke
National Cancer Institute, Frederick, MD, USA

Background: There are at least four distinct resistance pathways that emerge against second-generation INSTIs: G118R, G140A/S + Q148H/K/R, N155H, and R263K. Although it is critical to develop INSTIs that retain potency against all four resistance pathways, an important aspect of our research has been directed at overcoming resistance associated with G140S/Q148H mutants, a pathway common in PLWH who have undergone virological failure. We are using combinations of our most promising compounds to develop new INSTIs with improved antiviral potencies against the IN G140S/Q148H mutants.

Methods: Compound AK-01 was generated by combining some of the best structural features of our previous compounds 4d and 5j to produce a naphthyridine scaffold with modifications at the 4- and 5- positions. Single round infectivity assays were used to measure EC50 values against a panel of clinically relevant IN mutants. We compared the antiviral potencies of AK-01 with the potencies of parent compounds, 4d, 5j, another naphthyridine-based prototype INSTI (4a), and FDA-approved second-generation INSTIs.

Results: AK-01 exhibited high antiviral potencies against WT HIV-1 (1.2 ± 0.4 nM) and several well-characterized IN mutants (G118R = 3.8 ± 0.7, N155H = 2.3 ± 0.2 nM, and R263K = 2.8 ± 0.6 nM). Importantly, AK-01 showed only a modest loss in potency against the key IN mutant G140S/Q148H (17.3 ± 4.2 nM). However, there was a more pronounced loss of antiviral potency when AK-01 was challenged with some other well-known IN double and triple mutants.

Conclusion: Although AK-01 is not a fully optimized compound, our results show that combining structural modifications of promising naphthyridine-based INSTIs can be used to produce new compounds that are effective against some of the known drug-resistant mutants. Importantly, AK-01 had an improved antiviral profile when compared to cabotegravir. We are currently using the available structural and virological data to design and evaluate additional INSTIs.

636 Preclinical Characterization of GS-5894, a Potent NNRTI With Once-Weekly Oral Dosing Potential
Eric Lansdon, Andrew Mulata, Peti Jansal, Gary Lee, George Stepan, Mike Matles, Kelly Wang, Carmen Ip, Julie Fogarty, Dan Snavool, Bernard Murray, Stephen Yant, Zlatko Janeba, Richard L. Mackman, Tomas Chiar
Gilead Sciences, Inc, Foster City, CA, USA, Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic

Background: Non-nucleoside reverse transcriptase inhibitors (NNRTIs) target an allosteric binding pocket near the polymerase active site of HIV-1 reverse transcriptase (RT) to prevent viral replication. To date, all approved NNRTIs must be dosed once-daily as part of a highly active antiretroviral regimen. Due to their physiochemical properties such as high solubility and high logD, NNRTIs are good candidates for long-acting regimes. Here we describe a novel and potent NNRTI with metabolic stability supportive of once-weekly (QW) oral dosing for the treatment of HIV-1 infection.

Methods: Antiviral activity against HIV WT virus and drug resistant variants was measured in cytopathic assays using the MT-2 and MT-4 cell lines. Cross-resistance was assessed against a Monogram panel of HIV-1 reporter viruses containing NNRTI resistance-associated mutations. Mutations that emerged under selective drug pressure were identified by dose-escalation and fixed-drug concentration resistance selections. Compound binding to rat, dog and human plasma was measured by equilibrium dialysis. Predicted clearance (CL) was measured by metabolic stability in human hepatocytes. Additional oral and intravenous pharmacokinetic studies were conducted in rat and dog.

Results: GS-5894 is a potent and selective inhibitor of HIV-1 replication in the MT-4 cell line, in primary human CD+ T lymphocytes, and in monocyte-derived macrophages, with EC50 and selectivity index (EC50/EC50) ranging from 1.5 to 4.2 nM and 5,152 to >66,000, respectively. The antiviral activity of GS-5894 against a panel of 32 NNRTI-resistant reporter HIV-1 variants derived from treatment-experienced patients with HIV (PWHA) was superior to that of other marketed NNRTIs. GS-5894 dose escalating resistance selections.
resulted in the emergence of an HIV-1 triple RT variant (I125V+I138K+P236T) that was cross-resistant to EFV and RPV. GS-5894 is tightly bound to plasma across species though the human binding-adjusted EC50 is 122 nM. The human predicted CL for GS-5894 (uncorrected for plasma binding) is 0.17 L/h/kg. GS-5894 has oral bioavailabilities of 34% and 31% (dosed as a solution), and mean-residence times of 2.9 and 23 hours in rat and dog, respectively.

**Conclusion:** GS-5894 is a novel and potent NRTTI with an improved resistance profile compared to other NRTTIs. Given the high plasma binding and low predicted metabolic clearance, GS-5894 has the potential for once-weekly oral dosing in PWH for the treatment of HIV-1 infection.

### 638 Discovery of GS-9770: A Novel Unboosted Once Daily Oral HIV Protease Inhibitor

Xiaochun Han, Ron Aoyama, Jacob Cha, Aesop Cho, Ana Z. Gonzalez, Salman Jabri, Michael Lee, Albert C. Lilicic, Ryan McFadden, Andrew Mulato, Zach E. Newby, Jie Xu, Johannes Voigt, Lianhong Xu, Hong Yang, Guohai Scowens, Inc, Foster City, CA, USA

**Background:** HIV protease is one of the first biochemical targets identified to inhibit HIV replication. Nine HIV-protease inhibitors (PIs) have been approved since the first, saquinavir, in 1995. The latest showing high efficacy and barrier resistance. However, these agents have poor metabolic stability and a short human half-life on their own. As a result, HIV-PIs need to be co-administered with a CYP-inhibitor or pharmacokinetic (PK) booster, such as Cobicistat, to extend their half-life long enough to achieve daily oral dosing, hampering their broad utility.

**Methods:** To meet this need, it was our goal to discover a novel HIV-PI that could be administered without the need of a PK booster. Attributing the poor metabolic stability of known HIV protease inhibitors to their peptidomimetic nature and inspired by the guanidine scaffold which was used in the discovery of non-peptidomimetic, and unboosted, β-secretase 1 (BACE1) inhibitors, we selected an iminohydantoin phosphomonoester to initiate a structure-enabled optimization to GS-9770.

**Results:** GS-9770 is exquisitely potent (K<sub>i</sub> = 0.14 nM, EC<sub>50</sub> = 7 nM) and metabolically stable (SH HLM pred CL = 0.09 L/h/kg). Pre-clinical in vivo PK studies showed that GS-9770 had good oral bioavailability (46 – 100 %) and long half-life (7 – 12 hours).

**Conclusion:** Both in vitro and in vivo data of GS-9770 support its unboosted, once daily oral administration. This poster will walk audience through our medicinal chemistry efforts with structure-based drug design approach towards GS-9770.

### 639 Discovery of MK-8527: A Long-Acting HIV-1 Nucleoside Reverse Transcriptase Translocation Inhibitor

Izzat Raheem, Kerry Fillgrove, Gregory O'Donnell, Jonathan Patteson, Shih Lin Goh, Carolyn Bankhead-Tsits, Qian Huang, Ernest Asante-Appiah, Min Xu, Steve S. Carroll, Jay A. Grobler, Ana Z. Gonzalez, Vinay Girijavallabhan, Tracy L. Diamond, Merck & Co, Inc, Rahway, NJ, USA

**Background:** Nucleoside reverse transcriptase transcriptase inhibitors (NRTTIs) such as islatravir (ISL) are potent inhibitors of HIV-1 replication. We have invented a novel NRTTI with antiviral potency and pharmacokinetics (PK) suitable for less-frequent-than-daily dosing, an attractive profile for HIV pre-exposure prophylaxis. MK-8527 is a 7-deaza-deoxyadenosine analog and is phosphorylated intracellularly to its active triphosphate (TP) form, which is a potent inhibitor of HIV-1 replication.

**Methods:** MK-8527 was discovered through a lead optimization campaign focused on identifying structurally and functionally novel NRTTIs with the potential for extended-duration dosing. The mechanism of MK-8527-TP was evaluated in primer extension and footprinting assays, and antiviral activity and persistence after washout were measured in cell-based assays. PK parameters were evaluated in rats and Rhesus monkeys. Off-target activity was assessed against human DNA polymerases and in a panel of 114 enzyme/receptor binding assays.

**Results:** Systematic evaluation of key positions around the nucleoside core confirmed steep SAR associated with this compound class, particularly at the 2', 3', and 4' positions. Nucleobase modifications were tolerated, and a thorough evaluation of this and other positions led to the discovery of MK-8527. MK-8527-TP inhibits reverse transcriptase by immediate (translocation) and delayed chain termination. MK-8527 has comparable antiviral activity (human PBMC IC<sub>50</sub> = 0.21 µM) and persistence of antiviral effect after washout to ISL.

The PK of MK-8527 in rats and monkeys was characterized by low to moderate clearance and volume of distribution, with good oral absorption. Following oral administration of MK-8527 to monkeys, the TP had an intracellular half-life of 7.1 h, significantly longer than the plasma t<sub>1/2</sub> of the parent, of the parent, MK-8527 (7 – 11 h), as observed with other nucleos(t)ide analogs. MK-8527-TP displayed IC<sub>50</sub> values of ≥55 µM against the human DNA polymerases tested. Neither MK-8527 nor MK-8527-TP exhibited off-target activities at 10 µM in the panel of enzyme/receptor binding assays tested.

**Conclusion:** The subnanomolar potency, absence of off-target activity, and suitable PK for at least once-weekly dosing make MK-8527 an attractive clinical candidate for prophylaxis of HIV-1 infection.

### 640 High-Dose VH3810109 (N6LS) ± Recombinant Human Hyaluronidase PH20: Phase I Study Safety Results

**Methods:** SPAN (ICTS5291520) is a phase I, open-label, 3-part study assessing safety, tolerability, and pharmacokinetics of single-dose N6LS in healthy adults. Part 1 evaluated N6LS 20 mg/kg SC + rHuPH20 2000 U/mL, part 2 N6LS 60 mg/kg IV, and part 3 N6LS 3000 mg SC + rHuPH20 2000 U/mL. Adverse events (AEs), injection site reactions (ISRs), vital signs, electrocardiograms, and clinical laboratory values were monitored for 24 weeks.

**Results:** Eight participants were enrolled in each part to receive a single dose of N6LS. In the SC N6LS + rHuPH20 dose groups (parts 1 and 3), no relevant differences in overall AE incidences were observed (Table); across parts, no AEs led to withdrawal. In parts 1 and 3, among 32 ISRs reported by 15/16 (94%) participants, 17 were grade 3 (all injection site erythema based on size; mean duration: 2.9 days [part 1] and 5.7 days [part 3]; Table). All ISRs resolved without sequelae within ≤7 days except for 1 in part 3 that resolved after 27 days. Biphasic injection site erythema was reported in parts 1 (2/8 [25%]) and 3 (4/8 [50%]). Most participants rated local reactions and pain as acceptable and were in favor of injection treatment. No local secondary infections were reported with ISRs. In part 2 (IV), 1 participant experienced 2 grade 1 AEs; no ISRs were reported. A higher frequency of AEs was reported with SC administration compared with IV, mainly driven by ISRs. Across doses, no serious AEs or deaths were reported. A higher frequency of AEs was reported with SC administration compared with IV, mainly driven by ISRs. Across doses, no serious AEs or deaths were reported. A higher frequency of AEs was reported with SC administration compared with IV, mainly driven by ISRs. Across doses, no serious AEs or deaths were reported.

**Conclusion:** High-dose N6LS, when given IV or SC + rHuPH20, was generally safe and well tolerated in this study. These results support the ongoing clinical development of N6LS 3000 mg SC + rHuPH20 and N6LS 60 mg/kg IV into phase IIb.
A Dose Escalation Study of Safety & PK of TMB-365 & TMB-380 in People With Suppressed HIV
Jacob P. Lalezari,1 Moti Ramgopal,2 Gary Richmond,3 Gordon Crofoot4, Kuei-Ling Kuo,5 Yingan Lai,6 Martin Markowitz7
1Quest Clinical Research, San Francisco, CA, USA, 2Midway Immunology and Research Center, Fort Pierce, FL, USA, 3CAN Community Health, Fort Lauderdale, FL, USA, 4Coforthy Research Center, Houston, TX, USA, 5TiMed Biologics Inc, Taipei City, Taiwan, 6TiMed Biologics Inc, Irvine, CA, USA

Background: TMB-365, a second generation post-attachment bNAb, binds to the second domain of CD4 and is designed to display improved PK, antiviral activity, and breadth of coverage when compared toibalizumab. TMB-380 (aka VRC07-523LS) is a bNAb that binds to the CD4 binding site of HIV Env and has potent antiviral activity and favorable safety and PK profile. The combination is designed as a complete regimen for HIV therapy. Here we present available safety and PK results of the combination given as a single IV infusion to inform the feasibility of maintenance therapy given every 8-12 weeks in suppressed HIV-infected individuals.

Methods: Three groups of 10 subjects were infused with 2400 mg (Group 1), 3200 mg (Group 2), or 4800 mg (Group 3) of each bNAb in 250 ml NS over one hour and followed for 12 to 16 weeks. Groups were dosed sequentially with dose escalation approved by an independent Data Monitoring Committee. Participants were suppressed with continuous oral cART for at least 6 months, clinically stable, without a history of severe allergies, and had no history of virologic failure on previous treatment regimens. All infusions were performed over 60 minutes in outpatient clinics with vital sign determinations every 15 minutes during and up to 3-5 hours post-infusion. A subset of participants was pre-medicated with acetaminophen 650 mg and cetirizine 10 mg at the investigator’s discretion. Trough PK targets were ≥0.3 μg/ml and 65 μg/ml for TMB-365 and TMB-380, respectively in at least 80% of participants in any treatment group.

Results: All 30 subjects completed the study. One Group 3 subject was erroneously dosed with 1600 mg of each antibody and is excluded from PK analysis. No SAES, Grade 3 or 4 adverse events, or acute infusion events were attributed to infusions with bNAb. 2 subjects experienced delayed onset of fatigue and chills interpreted as hypersensitivity. PK data is shown below. Duration of concentrations above trough targets is increased with increased dose. In Group 3 participants at week 8, mean TMB-365 and TMB-380 concentrations were 15.1 and 86.4 μg/ml, respectively, and approximately 80% of participants met pre-defined trough targets.

Conclusion: A single infusion of TMB-365 and TMB-380 in combination up to 4800 mg each is safe. Prolonged PK duration was observed for both TMB-365 and TMB-380 and results suggest that an every 8-week infusion is feasible and will be tested in a Phase 2 clinical study.

Switching From a Second-Line Boosted PI Regimen to B/F/TAF: Results of a Randomized Clinical Trial

Samuel Pierre1, Jean Bernard Marc1, Fabienne Homeus1, Guillaume Rivette Bernadin1, Letizia Treviti1, Evens Jean1, Emelyne Dumont1, Sanjana Sundaresan1, Vanessa Rivera1, Dennis Israelski1, Sean E. Collins1, Jean W. Pape1, Patrice Severin1, Paul E. Sax1, Serena Koenig1
1GHESKIO, Port-au-Prince, Haiti, 2Harvard Medical School, Boston, MA, USA, 3Analysis Group, Inc, Boston, MA, USA, 4Gilead Sciences, Inc, Foster City, CA, USA, 5Brigham and Women’s Hospital, Boston, MA, USA

Background: Patients on second-line boosted PI-based regimens in resource-limited settings have high rates of NRTI resistance, but this information is unknown in routine clinical care. This study compared continuing boosted PI + NRTIs vs bictegravir/tenofovir alafenamide/emtricitabine (B/F/TAF) in PWH on second-line ART with no prior drug resistance testing.

Methods: This prospective, open-label trial conducted at GHESKIO in Port-au-Prince, Haiti, randomized adults (>21 years) with viral suppression on second-line PI/r-based ART to continue their current regimen vs. switch to B/F/TAF. The primary endpoint was the proportion of participants with HIV-1 RNA ≥200 copies/ml at week 48 using the FDA snapshot algorithm, the difference between groups was assessed with a non-inferiority margin of 4%.

Results: Between October 2020 and March 2023, 290 participants were randomized and treated (B/F/TAF: 149; bPI: 141). Median age was 50 years (IQR 42, 56) and 65% (57%) were women. At enrollment, 175 (60%) were taking lopinavir/r and 115 (39%) atazanavir/r; 226 (78%) were taking tenofovir disoproxil fumarate, 51 (18%) zidovudine, and 13 (4%) abacavir; all were taking lamivudine or emtricitabine. The median time on PI/r was 3.7 years (IQR 2.2, 5.7) years. At week 48, the proportion with HIV-1 RNA <200 copies/mL was 0.7% (1/149) and 2.8% (4/141) in the B/F/TAF and PI/r groups, respectively: difference -2.1 (95% CI: -6.7 to 1.2), meeting non-inferiority for B/F/TAF compared to PI/r (Table 1).

Table 1. Primary Endpoint – Virologic Outcomes at Week 48

<table>
<thead>
<tr>
<th>Week 48 Outcome</th>
<th>B/F/TAF (n=149)</th>
<th>Boosted PI (n=141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary endpoint: HIV-1 RNA &lt;200 copies/mL</td>
<td>1 (0.7%)</td>
<td>4 (2.8%)</td>
</tr>
<tr>
<td>HIV-1 RNA &lt;200 copies/mL in 48-wk window</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Treatment discontinued before wk 48 owing to lack of efficacy</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Died or LTFU with last available HIV-1 RNA value of ≥200 copies/mL</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HIV-1 RNA &lt;200 copies/mL in 48-wk window</td>
<td>148 (94.9%)</td>
<td>129 (91.5%)</td>
</tr>
<tr>
<td>No data for final outcome (censored)</td>
<td>8 (5.4%)</td>
<td>8 (5.7%)</td>
</tr>
</tbody>
</table>

Conclusions: Switching virally suppressed adults on a second-line PI/r regimen to B/F/TAF is non-inferior to continuing PI/r-based ART. Rates of viral suppression were high in both groups.
Phase II Study of Switch to Daily BIC + LEN in Individuals on a Multitablet HIV Treatment Regimen

Karam Mounzer1, Jihad Slim2, Moti Ramgopal3, Malcolm Hedgcock1, Mark Bloch4, Jorge Santana4, Ines Mendes4, Ying Guo5, Priyanka Arora6, Jairo M. Montezuma-Rusca1, Hal Martin1, Peter Sklar7, Jared Baeten8, Sorana Segal-Maurie5
1Haddow FGH South, Philadelphia, PA, USA, 2University of New Mexico College of Osteopathic Medicine, Valhalla, NY, USA, 3Midway Immunology and Research Center, Fort Peace, IL, USA, 4Spectrum Health, Vancouver, Canada, 5Coldstream House Medical Practice, Darlington, Australia, 6University of Puerto Rico, San Juan, Puerto Rico, 7Galde Sciences, Inc, Foster City, CA, USA, 8New York Presbyterian Hospital Queens, New York, NY, USA

Background: While single-tablet regimens (STRs) are currently the global standard for HIV treatment, some people with HIV (PWH) take multi-tablet regimens (MTR) due to treatment resistance, intolerance or drug interactions. The combination of bictegravir (BIC), an integrase strand transfer inhibitor, and lenacapavir (LEN), a first-in-class capsid inhibitor, could simplify treatment in virologically suppressed (VS) PWH for whom STRs are not indicated. We report the Phase 2, 24-Week primary outcomes for BIC + LEN versus stable baseline regimen (SBR) in VS PWH on a complex regimen.

Methods: ARTISTRY-1 (NCT05502341) is an ongoing, randomized, open-label, multicenter Phase 2/3 study. In Phase 2, 128 participants on SBR (≥6 months prior to screening) were randomized 2:2:1 to receive once-daily oral BIC 75 mg + LEN 25 mg, oral BIC 75 mg + LEN 50 mg or continue SBR. All participants receiving BIC + LEN received an oral loading dose of LEN 600 mg on Days 1 and 2 of treatment. The primary endpoint was the proportion of participants with HIV RNA ≤50 copies/mL (FDA Snapshot) at Week 24. Secondary endpoints included the proportion of participants with HIV RNA <50 copies/mL, change from baseline in CD4 cell count, and the proportion of participants with treatment emergent adverse events (TEAEs) up to Week 24.

Results: 51 and 52 participants received BIC 75 mg + LEN 25 mg or BIC 75 mg + LEN 50 mg, respectively, and 25 continued SBR. At baseline, 19% of participants were female, 31% were Black and 16% were Hispanic or Latinx; median (Q1, Q3) age was 60 (56, 65) years and participants were taking a median (range) of 3 (2–9) tablets per day. Outcomes at Week 24 are shown in the Table. HIV-1 RNA was ≤50 copies/mL in 0/51 of participants in the BIC 75 mg + LEN 25 mg group, 1/52 (2%) in the BIC 75 mg + LEN 50 mg group (later suppressed to <50 copies/mL without regimen change) and 0/25 in the SBR group. CD4 counts were comparable in all groups. The most common TEAEs in the two BIC + LEN treatment groups up to Week 24 were diarrhea (7%), COVID-19 (6%) and constipation (5%). Drug-related TEAEs occurred in 18%, 6% and 0% of participants, respectively.

Conclusion: BIC + LEN was highly effective in maintaining viral suppression in participants switching from an MTR, with similar safety profiles observed in the two BIC + LEN treatment groups. These data support the use of BIC and LEN in combination to simplify treatment in VS PWH who are receiving complex regimens. A BIC/LEN STR will be tested in the Phase 3 part of the study.

Impact of Switching From Dual to Triple Therapy on Inflammation: INSTINCT Study

Sergio Serrano-Villar1, Laura Martin-Pedraza2, Carmens Busca1, Juan Manuel Tiraboschi3, Jesús Santos4, Luis Fernando López-Cortés5, Carmen Hidalgo Tenorio6, Vicente Estrada7, María José Crusellas Canals8, Alfonso Cabello Ubeda9, Alberto Díaz de Santiago10, Anton L. Pozniak11, Miguel Torralba12, Marta De Miguel13, Santiago Moreno14,15
1Hospital Ramón y Cajal, Madrid, Spain, 2La Paz University Hospital, Madrid, Spain, 3Hospital Universitario de Bellvitge, Barcelona, Spain, 4Hospital Universitario Virgen del Rocío, Sevilla, Spain, 5Hospital Universitario Virgen de las Nieves, Granada, Spain, 6Hospital Universitario Clínico San Carlos, Madrid, Spain, 7Hospital Clinico Universitario La Fe, Valencia, Spain, 8Fundacion Jimenez Diaz, Madrid, Spain, 9Hospital Puerta de Hierro, Madrid, Spain, 10Hospital Universitario Príncipe de Asturias, Madrid, Spain, 11Hospital Universitario de Guadalajara, Guadalajara, Spain, 12Fundación SEIMC-GeISDA, Madrid, Spain

Background: Because inflammation is associated with mortality and has been linked to HIV transcription in lymphoid tissues during ART, it is necessary to address the long-term effects of switching ART regimens with different numbers of antiretrovirals on inflammation.

Methods: In this interim analysis of the randomized, open-label, multicenter INSTINCT trial (clinicaltrial.gov: NCT04076423), we evaluated the effect of switching from DTG/3TC to BIC/FTC/TAF vs. remaining on DTG/3TC on systemic inflammation up to 48 weeks. Participants were adults with confirmed, virologically suppressed HIV, on stable ART with DTG/3TC for a minimum of 48 weeks. Exclusions included previous virological failure, drug resistance, and autoimmune conditions. We focused on IL-6 changes from baseline to week 48.
Results: A total of 118 patients with available week 0 and 48 plasma samples were included; 17% women, mean age 44 ± 11 years, 79% white, 62% MSM, 9% with past AIDS diagnosis, median nadir CD4 338 cells/µL, baseline CD4 counts 776/µL, median duration of HIV suppression 5.2 years, median IL6 levels within normal range (0.96 pg/mL [0.53-1.44]). No differences were observed in the general characteristics between groups. No significant differences in IL-6 changes were observed between groups from week 0 to 48 (median fold change for DTG/3TC, 1.1 [0.7-1.7]; for BIC/FTC/TAF, 1.2 [0.8-1.8], P = 0.688) (Figure 1). Multivariate linear regression analyses corroborated these findings (Coef -0.07, P = 0.031).

Conclusion: This analysis revealed no significant impact on IL-6 levels when switching from DTG/3TC to BIC/FTC/TAF after 48 weeks. These preliminary findings suggest a neutral inflammatory effect for the ART switch, warranting further study to elucidate the longer-term influence on inflammation.

647 Impact of Switch to 3TC/DTG on the HIV-1 Transcriptional Reservoir in a Randomized Controlled Trial
Evy E. Blomme1, Evelien De Smet1, Mareva Delporte1, Wim Tryptsteen1, Sophie Degroote1, Sophie Vanherweghe1, Eli Caluwé1, Marie-Angélique De Scherder1, Linos Vandenkerckhove1
1Ghent University, Ghent, Belgium
2Ghent University Hospital, Ghent, Belgium

Background: The Rumba study is the first randomized clinical trial evaluating the impact on the viral reservoir of switching from a 2nd generation integrase inhibitor (INI)-based triple ART regimen towards 3TC/DTG vs. B/F/TAF. We observed no differences in the dynamics of the total and intact HIV-1 DNA reservoir after 48 weeks. Here, we quantified the mean change from baseline at 48 weeks of different HIV-1 RNA transcripts as a secondary objective, to ensure switching to 3TC/DTG does not increase transcriptional activity.

Methods: In this prospective controlled switch trial, participants with HIV-1 RNA<50 copies/mL plasma at least 3 months on any stable 2nd generation INI-based triple ART were randomized to switch to 3TC/DTG (N=89) or to switch or stay on B/F/TAF (N=45). HIV-1 transcripts that define distinct blocks to transcription were analyzed on CD4 T cell cDNA of subtype B participants in duplicate. An in-house 4-plex Rainbow transcriptional RNA dPCR assay for long LTR, pol, polyA and tat-rev was run on the QIAseqy system. RNA concentrations were used to normalize HIV-1 RNA transcripts. Statistical analysis was performed with an ordinary linear regression model on log-transformed data, adjusted for baseline response value, CD4 nadir and time on ART. Data analysis was performed with an in-house R script, a modified version of dPCRquant to accommodate QIAseqy system data analysis.

Results: Baseline characteristics are reported in Table 1. In this complete case analysis (3TC/DTG n=52; vs. B/F/TAF n=23), the mean baseline copy number per µg RNA was 207.7 for long LTR (elongated transcripts), 24.5 for pol (unspliced transcripts) and 77.9 for polya (completed transcripts), suggesting a block to completion of transcription. Tat-rev (multiple-spliced transcripts, surrogate for productive infection) was undetectable in the majority of the samples at both timepoints. After 48 weeks, the relative change from baseline of elongated, unspliced and completed HIV-1 RNA transcript copies was not significantly different between 3TC/DTG and B/F/TAF (1.11 [0.78-1.56], 0.83 [0.55-1.25] and 1.17 [0.85-1.61], respectively, point estimates and 95% CI). Also, within the treatment arms, no evidence of a significant mean difference over time was observed.

Conclusion: There is no evidence that simplification of a triple ART INI-based regimen to 3TC/DTG impacts the overall size of the reservoir, neither the fraction of intact virus nor the transcriptional activity.

Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th>Randomized participants</th>
<th>Total N=131</th>
<th>B/F/TAF N=45</th>
<th>3TC/DTG N=86</th>
<th>3TC/DTG vs B/F/TAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT population</td>
<td>135</td>
<td>45</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>58/77</td>
<td>23/22</td>
<td>32/64</td>
<td></td>
</tr>
<tr>
<td>Ethnicity (European/African/other)</td>
<td>102/14/13</td>
<td>39/7/8</td>
<td>63/5/9</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>47 (30-59)</td>
<td>47 (30-57)</td>
<td>47 (30-56)</td>
<td></td>
</tr>
<tr>
<td>CD4 count (cells/µL)</td>
<td>589 (456-854)</td>
<td>583 (450-850)</td>
<td>617 (532-973)</td>
<td></td>
</tr>
<tr>
<td>Time on ART (y)</td>
<td>7.2 (6.0-8.8)</td>
<td>7.6 (6.8-8.9)</td>
<td>6.6 (5.8-7.5)</td>
<td></td>
</tr>
</tbody>
</table>

*ITT-6: intention-to-treat exposed

645 Switching to DTG+3TC vs 3-Drug Regimens in Routine Clinical Care: Long-Term Swedish Data
Erik Sörstedt1, George Nduva1, Saara Hiltunen1, Johanna Repts1, Frederik Månsson1, Åsa Mellgren1, Eva Ferm1, Adam Stubbs1, Melanie Schroeder1, Johanna Brännström1, Christina Carlander1
1University of Gothenburg, Gothenburg, Sweden, 2GlasgowSmithKline, Stockholm, Sweden, 3IBCH Medical, Stockholm, Stockholm, Sweden, 4Lund University, Lund, Sweden, 5HIV Healthcare, Brentford, United Kingdom, 6Karolinska Institute, Stockholm, Sweden

Background: Swedish HIV treatment guidelines recommend switching to the 2-drug regimen dolutegravir (DTG) + lamivudine (3TC) for maintaining virologic suppression should be considered in people living with HIV (PHLV) in routine clinical care. We assessed long-term outcomes of switching to DTG+3TC (vs 3-drug regimens, 3DR) in virologically suppressed individuals in Sweden.

Methods: Retrospective data of all ART-experienced PHLV with HIV RNA<50 copies/mL and switching to either DTG+3TC or 3DR were obtained from the Swedish National Registry for HIV (InfCareIVH). Within-group precision rates for virological failure (VF, having two consecutive HIV RNA levels ≥200 copies/mL prior to/ by assessment timepoint) were determined for DTG+3TC and 3DR groups at months M6, M12, M24, M36, and M42 post-switch. A logistic generalized estimating equations (GEE) model was used to find associations between patient demographic and clinical predictors on VF.

Results: 2461 PHLV switched regimen between July 2019 – May 2023; 1125 (46%) to DTG+3TC and 1336 (54%) to 3DR. The mean estimated time of switching to DTG+3TC and 3DR groups at months M6, M12, M24, M36, and M42 post-switch. In a GEE model was used to find associations between patient demographic and clinical predictors on VF.

Changes in IL-6 plasma concentrations after 48 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-6 levels fold change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTG+3TC</td>
<td>(n = 60)</td>
</tr>
<tr>
<td>0.1</td>
<td>1.0-9.0</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0-9.0</td>
</tr>
<tr>
<td>2.0</td>
<td>1.0-9.0</td>
</tr>
<tr>
<td>3.0</td>
<td>1.0-9.0</td>
</tr>
<tr>
<td>4.0</td>
<td>1.0-9.0</td>
</tr>
</tbody>
</table>

646 Impact of Switching to DTG+3TC vs 3-Drug Regimens Based on Viral Markers: For First-Line ART
Jose Molto1, Maria C. Garcia-Guerrero1, Luna Bailor1, Igor Moraes-Cardoso2, Ester Aparicio2, Pep Coll2, Angel Rivero2, Elias P. Rosen3, Jacob D. Estes4, Julia Blancon5, Alex Olivera1, Maria C. Puerta1, Beatriz Mothe1, Javier Martinez-Picado1, for the DUALITY Study Group
1Hospital Germans Trias i Pujol, Barcelona, Spain, 2Pepinstitute for AIDS Research, Badalona, Spain, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 4Viemgen Health and Sciences University, Portland, OR, USA

Background: Data on the effect of dual therapy with DTG/3TC as first-line ART on the evolution of the HIV-1 reservoir and inflammatory biomarkers are lacking. Our objective was to compare the effect of first-line ART with DTG+3TC versus one standard 3-drug regimen (3DR) on the dynamics of viral persistence in lymph nodes and peripheral blood and on immune activation biomarkers during the 1st year after ART initiation. Here, we present data on the evolution of HIV persistence and immune-associated parameters in peripheral blood.
Methods: DUALITY is a 48-week, single-centre, randomized, open-label clinical trial in ART-naïve people with HIV (PWH). Participants were randomized (1:1) to receive DTG+3TC (2DR group), or with DTG+FTC/TAF (3DR group). Total and intact proviral HIV-1 DNA and cell-associated RNA (ca-RNA) in CD4+ T cells were longitudinally determined by ddPCR. The inducible reservoir was measured as the frequency of HIV-infected CD4+ T cells able to produce p24 by the VIP-SPOT assay. Soluble inflammatory markers (IL-6, sCD14, TRAIL, IP10, FABP2, CRP and D-dimer) were measured by ELISA. Activation (HLA-DR/CD38) and exhaustion markers (PD-1/TIGIT) in CD4+ and CD8+ T cells were determined by multiparametric flow cytometry.

Results: Forty-two participants were included (22 per study arm). At baseline, mean (SD) log10 pVL, CD4+ and CD8+ T cell count were 4.4 (0.7) copies/mL and 493 (221) cells/mm3. Two participants were lost, and one withdrew informed consent before week 48. All participants completing the study (2DR n=20; 3DR n=21) had pVL <50 copies/mL at week 48 except one in the 3DR group who was resuppressed after treatment for syphilis. Changes from baseline to week 48 in all reservoir parameters were similar between 2DR and 3DR groups (Table). At week 48, levels of total and intact proviruses were similar between both groups, and correlated with pre-ART pVL (Rho 0.50, p=0.002 for intact HIV-1 DNA). Changes in soluble inflammatory biomarkers and in levels of activated/exhausted CD4+ and CD8+ T cells were also comparable between study groups. Complementary data on anatomic distribution of antiretroviral drugs and viral expression in lymph nodes are under study.

Conclusion: First-line dual ART with DTG+3TC resulted in a similar decay in parameters of HIV-1 persistence in periphery as well as in immune-associated markers compared to 3DR with DTG+FTC/TAF. Our results further support recommendations of DTG/3TC as one preferred option for first-line ART in PWH.

Table: Median (min-max) fold change from baseline to week 48 in HIV-1 reservoir parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2DR Group</th>
<th>3DR Group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DNA</td>
<td>0.81 (0.33-0.93)</td>
<td>0.82 (0.50-0.94)</td>
<td>0.46</td>
</tr>
<tr>
<td>Intact DNA</td>
<td>0.82 (0.50-0.95)</td>
<td>0.84 (0.52-0.97)</td>
<td>0.62</td>
</tr>
<tr>
<td>ca-RNA</td>
<td>0.93 (0.76-1.00)</td>
<td>0.94 (0.81-1.00)</td>
<td>0.90</td>
</tr>
<tr>
<td>VIP-SPOT</td>
<td>0.97 (0.78-1.00)</td>
<td>0.96 (0.69-1.00)</td>
<td>0.93</td>
</tr>
</tbody>
</table>

469 Uptake of Rapid and Early ART Initiation in Latin America and the Caribbean and Associated Factors

Yanick Caro-Vega1, Anna K. Person1, Bryan E. Shepherd2, Rodrigo Ville3, Serena Koenig4, Carina Cesar5, Claudia P. Cortes6, Beatriz Girmsztein7, Eduarto Gotuzzo1, Brenda E. Crabtree-Ramirez1

1Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, 2Vanderbilt University, Nashville, TN, USA, 3Harvard Medical School, Boston, MA, USA, 4Fundación Huésped, Buenos Aires, Argentina, 5University of Chile, Santiago, Chile, 6Fundación Cruz Fuentes - Facruz, Rio de Janeiro, Brazil, 7Universidad Peruana Cayetano Heredia, Lima, Peru

Background: In 2015, the WHO recommended initiation of ART in all persons with HIV (PWH) regardless of CD4 count. Same-day ART has proven to be feasible and effective in low- and middle-income countries and has become a standard of care in Haiti for those without TB symptoms since 2017. Nevertheless, implementation of starting ART rapidly represents a challenge in diverse settings. Thus, we evaluated the proportion of PWH with rapid and early ART initiation, associated factors and survival in our region.

Methods: We included PWH ≥18 years of age, diagnosed between 2017 and 2021, and presenting for care in CASANet sites. We estimated the proportion of those who initiated rapid ART (≤7 days; R-ART) and early ART (≤14 days; E-ART) after HIV diagnosis overall and within each site. We analyzed factors associated with E-ART (age, sex at birth, education level, mode of HIV acquisition, site, calendar year, CD4 cell count) with a logistic regression (excluding Haiti where R-ART is standard of care) and the association of E-ART with overall survival with adjusted Cox models.

Results: A total of 9173 PWH were included; 8507(92.7%) initiated ART, and of those 3146(37%) initiated R-ART and 4237(49%) E-ART. Overall, most of R-ART and R-ART starters were from Haiti: 2759(88%) and 4237(49%), respectively. Among those included on each site, the proportion of E-ART were: Argentina 3%, Chile 5.4%, Peru 6.8%, Mexico 25%, Brazil 34%, Honduras 44%, and Haiti 79%. Excluding Haiti, more recent year was associated with a higher probability (aOR[95%CI]) of E-ART (1.24[1.93-2.12]) in 2018 and (2.47 [2.25-2.62]) in 2021 vs 2017. Additionally, being female (1.71[1.15-2.85]), other mode of acquisition vs heterosexual mode (1.67[1.19-2.35]), upper secondary vs primary school (1.16[1.05-1.28]), and age at diagnosis (0.95[0.90-0.99]) per each 10 additional years were associated with E-ART. Overall, E-ART was not associated with higher survival (aHR: 0.93[0.95-1.00], p=0.052 including Haiti; aHR: 0.90[0.85-0.97], p=0.01 excluding Haiti).

Conclusion: Despite of the recommendation of ART initiation as soon as possible, Haiti is the only site in CASANet to initiate ART early after diagnosis of HIV in most patients. Women, younger and more educated people were more likely to initiate E-ART. Heterogeneity between health systems, policies and differential characteristics of participant sites including linkage to care strategies may contribute to these results. More research to explain these findings, is needed.
Bictegravir as Universal Initial Antiretroviral Therapy: A National Target Emulation Study
Isaac Núñez1, Yanimik Caro-Vega1, Conor Macdonald1, Juan L. Mosquera2, Alicia Piñeirúa-Menéndez3, Anthony Matthews4, Serge Clotaire Billong1, Conor MacDonald5, Anthony Matthews6, Matthew A. Spinelli5, Jacques Kamangu3, Rachel S. Beard3, Juan L. Mosqueda1, Steven Y. Hong1, Samuel Martin Sosso1, Anne-Cecile Z-K Bissek3, Alexis Ndjolo3, Federico Perno4, Joanne Herriott, Edyta Kijak2, Alicja Piñeirúa-Mόndez3, Alexandra Hifindwako2, Assegid Mengistu2, Jacques Kamangu3, Gram Mutandi6, Daniella Mouton7, Fekir Negussie2, Rachel S. Beard3, Monica Gandhi3, Steven Y. Hong1, Joanne Sharp1, Megan Neary1, Manmohan Brahmbhatt2, Andrew N. Timmis4, Joanne Herriott, Edyta Kijak2, Daniela Mouton7, Fekir Negussie2, Rachel S. Beard3, Monica Gandhi3, Steven Y. Hong1, Joanne Sharp1, Megan Neary1, Manmohan Brahmbhatt2, Andrew N. Timmis4, Joanne Herriott, Edyta Kijak2, Daniela Mouton7, Fekir Negussie2, Rachel S. Beard3, Monica Gandhi3, Steven Y. Hong1.

**Background:** Bictegravir (BIC) has been shown to be non-inferior to dolutegravir (DTG) as initial antiretroviral therapy (ART) in non-inferiority randomized controlled trials (RCTs). Mexico established in 2019 a national policy to start ART with BIC to every ART naive person with HIV (PWHA). However, BIC has not been compared to efavirenz (EFV) or raltegravir (RAL) (commonly prescribed in Mexico) and no superiority trials have been performed.

**Methods:** We used data from the national antiretroviral surveillance system in Mexico to perform three target trial (TT) emulations for initial ART among PWHA starting ART from 2019 onwards: BIC vs DTG, BIC vs EFV, and BIC vs RAL. Our approach consists of specifying the protocol of an index TT (the RCT we would ideally conduct) and emulate it with observational data while adjusting for confounding to approach randomization. Baseline was the date of ART start. People with missing viral load (VL), VL <500 copies/mm^3, and/or missing CD4 count within the last year before ART start, those started on double-dose DTG and/or reduced-dose etravirine, lamivudine or tenofovir disoproxil fumarate were excluded. We performed individual logistic regressions while adjusting for treatment-outcome confounders (baseline VL and CD4, age, gender, imprisonment status, state of residence, year of ART start) to estimate risk of undetectable VL (UVL) (<50 copies/mm^3) for each person at 3 and 12 months. We performed intention-to-treat analyses. We calculated the mean risk of UVL for each group, risk ratios (RR) and 95% percentile-based confidence intervals using 500 bootstrap resamples.

**Results:** 26178 PWHA were included: 18894 started BIC, 2530 DTG, 562 RAL, and 4192 EFV. Most PWHA were cis men (85%), median age was 31 years. 57% had a baseline viral load <100k copies, and median CD4 cell count was of 213. Only 1% of PWHA were in a prison. At three months, the risk of UVL was of 79.2% vs 78.4% (BIC vs DTG, RR 1.01 [0.98-1.03]), 79.7% vs 71% (BIC vs RAL, RR 1.12 [1.03-1.21]), and 79.7% vs 63% (BIC vs EFV, RR 1.25 [1.21-1.3]). At twelve months, the risk of UVL was of 89.5% vs 83.6% (BIC vs DTG, RR 1.02 [0.98-1.08]), 85.9% vs 79% (BIC vs RAL, RR 1.08 [1.12-1.3]), and 86.6% vs 83% (BIC vs EFV, RR 1.04 [1.01-1.08]).

**Conclusion:** BIC is superior to RAL and EFV, but not DTG, as initial ART in treatment naive PWHA for reaching UVL at 3 and 12 months. These results support existing RCTs and argue in favor of this country-wide strategy.

HIV Detectable Low-Level Viremia Suggests a Revised Threshold for Viral Suppression in Cameroon
Alex Durand NKA1, Joseph Fokam1, Collin Ambe Chenwi2, Efakasi Gabisa Jeremiahia, Yaqui Boubi2, Serge Zoulahou Bilingou3, Anne-Cécile Z-Baisik2, Hamatsou Nadja1, Carlo Federico Perno4, Samuel Martin Sosso1, Alexis Ndjolo3, Centre International de Référence Chantel Bia, Yaoundé, Cameroon, University of Yaoundé, Yaoundé, Cameroon, Ministry of the Health Public Health of Cameroon, Yaoundé, Cameroon, Bambino Gesù Children’s Hospital, Rome, Italy

**Background:** Transitioning to dolutegravir-based therapy in Cameroon has improved viral suppression (VS) rates, known as low-level viremia (LLV) <1000 copies/ml. However, there is a growing number of patients experiencing LLV with 50-200 copies/ml increased significantly from 65.2% (534/819) in 2020, 70.7% (678/958) in 2021 and 72.2% (227/314) in 2022, p=0.001.

**Conclusion:** Even though VS rate appears encouraging, there is a significant increasing proportion of patients with detectable LLV in this DTG-era. Of note, UVL with 50-200 copies/ml appears highly predominant, suggesting a revision of threshold for VS at a maximum of 200 copies/ml in resource-limited settings like Cameroon.

Increased Viral Suppression With Adherence Counseling Incorporating a Point-of-Care Urine TVF Test
Leonard T. Bikinesi1, Matthew A. Spinelli2, Nombizodwa M. Nyoni3, Jessya Hifindwako2, Assegid Mengistu2, Jacques Kamangu3, Gram Mutandi6, Daniella Mouton7, Fekir Negussie2, Rachel S. Beard3, Monica Gandhi3, Steven Y. Hong1

1Ministry of Health and Social Services, Windhoek, Namibia, 2University of California San Francisco, San Francisco, CA, USA, 3UCSF Institute for Global Health Sciences – Namibia, Windhoek, Namibia, 4Namib Institute of Pathology, Windhoek, Namibia, 5US Centers for Disease Control and Prevention Windhoek, Windhoek, Namibia

**Background:** Innovative approaches are needed to achieve the third UNAIDS 95-95-95 target, to increase and sustain virologic suppression (VS) in patients on ART, specifically co-formulated tenofovir (TFV)-lamicudine-dolutegravir (DTG) or TLD. Virologic failure in patients on TLD is likely due to non-adherence because of DTG’s high inherent resistance barrier. Identification of adherence to TLD with a point-of-care (POC) metric and tailored counseling on the test may help patients achieve viral suppression (VS). We integrated a low-cost, POC urine test to detect TFV into standard WHO-recommended enhanced adherence counseling (EAC) to improve VS in adults with non-VS on TLD in Namibia.

**Methods:** Patients on TLD with viral load (VL) >1000 copies/ml after completing ≥1 round of EAC were enrolled from 42 clinics across Namibia. At each monthly ART pick-up, participants completed the POC urine test and received EAC informed by test results. After 3 months (round 1), participants received a viral load (VL) test. If VS was not achieved, up to 3 additional rounds of POC urine testing with EAC was provided, with an HIV drug resistance test sent at month(M) 9. Acceptability of the urine assay was assessed via surveys administered to participants and providers.

**Results:** Of 211 participants enrolled (median age 33 years, interquartile range 22-46, 61% female), 195 reached M3 and received a follow-up VL, with 169 (87%) achieving VS within M3 and 192 (93%) by M9. Moreover, in those who achieved VS, positive TFV in urine increased from 81% at baseline to 96% at M9 compared to a change from 31% to 41% among unsuppressed individuals. Drug resistance testing was performed in 5 remaining participants with high VL at M9. All 5 had variable urine TFV results over visits and one had DTG resistance (227/314) in 2022, p=0.001.

**Conclusion:** Nearly 90% of patients on TLD with VL >1000 copies/ml achieved VS within 3 months (93% at M9) following EAC that incorporated a urine-based POC TFV test, compared to 33% of individuals receiving 1-3 rounds of standard WHO-recommended EAC. Encouraging results of this pre-post intervention study show potential for the urine test to be effective in pre-exposure prophylaxis as a single agent and is available as a long-acting (LA) injectable medicine. This work describes preclinical pharmacokinetics of a novel BIC LA solid injectable.

Preclinical Pharmacokinetics of a Novel Long-Acting Bictegravir Solid Injectable in Rats
Usman Arshad1, Joanne Sharp, Megan Neary, Joanne Herriott, Edyta Kijak, Eduardo Gallardo-Toledo, Paul Curley, Helen Cox, Eleanor Barlow, James J. Hobson, Andrew B. Dwyer, Jonathan Massam, Steve Rannard, Andrew Owen University of Liverpool, Liverpool, United Kingdom

**Background:** Bictegravir (BIC), is an integrase strand transfer inhibitor, effective for HIV treatment when combined with emtricitabine and tenofovir alafenamide. Another integrase inhibitor, cabotegravir, has been demonstrated to be effective in pre-exposure prophylaxis as a single agent and is available as a long-acting (LA) injectable medicine. This work describes preclinical pharmacokinetics of a novel BIC LA solid injectable.
Methods: A solid drug nanoparticle (SDN) formulation of BIC was manufactured using emulsion-templated freeze-drying (ETFD) prior to formation of a solid format using vacuum compression moulding (VCM). Resulting BIC formulations were injected (16.8mg BIC per animal) subcutaneously with a 12-gauge needle into the scapular region of male Sprague Dawley rats (n = 4, 250-300g). Plasma samples were collected from the lateral tail vein for 13-weeks post injection. BIC concentrations were quantified in plasma using validated LC-MS/MS and the injection site was terminally harvested for histopathological analysis.

Results: ETFD yielded solids consisting of 70% BIC by weight, with subsequent dispersion in water yielding BIC particles with hydrodynamic diameters (Dz) between 700-850 nm, as determined by dynamic light scattering. Plasma BIC concentrations exceeded the human oral steady-state C_{ss95} within 3 hours of administration and remained above this level for 42 days (T_{1/2} = 2 days; AUC_{0-42} = 22427 μg h/mL). No behavioural issues were encountered, animals gained weight throughout and no visible injection-site reactions were evident.

Conclusion: Preclinical data for a novel BIC solid injectable demonstrated sustained therapeutic concentrations in rats for 42 days. Further work is required to understand dose linearity and proportionality, assess removability, and estimate doses and durations likely to be achievable in humans.

654 Shape-Shifting Tail Decay Is the Pharmacokinetic Profile of an Ultra-Long Acting Bictegravir Prodrug
Mohammad Ullah Nayan1, Ivana Massuf2, Srijane Das1, Brady Sillman, Brandon Hanson1, Arpan Acharya1, Tiancheng Edwards1, Charles W. Dobard2, Howard E. Gendelman1, Benson Edagwa1
1University of Nebraska Medical Center, Omaha, NE, USA, 2Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: The advantages of long-acting (LA) antiretroviral therapy (ART) for regimen adherence and sustained viral suppression are unquestioned. However, the requirement for bi-monthly clinic visits, injection site reactions, large dosing volumes, and long pharmacokinetic (PK) tail pose notable challenges to widespread LA ART use. Consequently, there is an immediate need for ultra-LA (ULA) ART capable of synchronizing dosing intervals with established six-month clinic visits. We report the PK and biodistribution profiles of two lead bictegravir (BIC) prodrug formulations in two animal species, in pursuit of a ULA BIC prodrug formulation with a short PK tail.

Methods: Dimeric (MXBIC) and monomeric (NM2BIC) BIC prodrugs were synthesized by esterification reactions and converted into aqueous surfactant stabilized solid drug nanosuspensions by high-pressure homogenization. Formulation stability, particle size, homogeneity, and surface charge were assessed. PK profiles, biodistribution, and terminal phase PK tails were evaluated in Sprague Dawley (SD) rats and rhesus macaques (RM) after intramuscular (IM) injection dosing.

Results: Dimeric BIC prodrug formulations (NMXbic formulations 1 and 2) demonstrated plasma BIC levels above the protein-adjusted 95% inhibitory concentration (PA-IC_{95}) for > 6 months in SD rats after a single 45 mg BIC eq./kg IM injection. Increasing the formulation concentration of NMXbic by 1.5 fold (NMXbic formulation 2) and subsequent reduction of injection volume boosted plasma BIC levels, but doubling the dose for formulation 2 in SD rats did not lead to a proportional increase in plasma BIC levels. Specifically, BIC levels following NMXbic formulation 2 dosing were sustained at levels ≥ 14x PA-IC_{95} for 6 months. In RM, a single IM injection of NMXbic (100mg, BIC eq./kg) sustained plasma BIC levels at ≥ 4x PA-IC_{95} for more than 6 months. Notably, regardless of the dose and species, plasma BIC levels after NMXbic treatment exhibited rapid drug decay after 7 months, demonstrating a short PK tail (Figure A-B). In contrast, the monomeric BIC prodrug formulation (NM2bic) exhibited a much slower BIC plasma decay curve after 7 months post-dosing in RM. An equivalent booster dose of NMZbic (50 mg, BIC eq./kg) in RM on day 217 resulted in higher plasma BIC levels than the corresponding time points after the first injection.

Conclusion: The dimeric NMXbic formulation exhibits high plasma exposure and a shorter PK tail with the potential for dosing at 6-month intervals.
Cabotegravir Stearate (XVIR-110), an InSTI Prodrug, Provides Ultra-Long Acting Cabotegravir Exposure

Brian P. Kearney, Brady Sillman, Howard E. Gendelman, Benson Edgawa, Leigh Ann Burns-Haas, Alborz Yazdi

Exavir Therapeutics, Omaha, NE, USA, University of Nebraska Medical Center, Omaha, NE, USA, Magnolia Toxicology Consulting, LLC, Traverse City, MI, USA

**Background:** Cabotegravir stearate (M2CAB) is a novel prodrug of the INSTI cabotegravir (CAB) that forms local and macrophage-distributed M2CAB depots, resulting in "flip-flop" plasma pharmacokinetics (PK) yielding a protracted apparent elimination half-life. M2CAB is formulated as an extended-release injectable suspension (XVIR-110) for intramuscular (IM) administration that may achieve ultra-long-acting therapeutic CAB exposures with infrequent administration.

**Methods:** We evaluated the PK profile of M2CAB and/or CAB in following a single IM (thigh muscle(s) of hind limb; 28-ga. syringe) administration of XVIR-110 to male SD rats and male beagle dogs. XVIR-110 was dosed at target M2CAB doses of 75, 185 (single and (2)-split injections) and 638 mpk (split injections) in rats and at 40 and 151 mpk in dogs. PK were assessed at multiple timepoints over up to 12 months until CAB concentrations fell to less than the apparent elimination half-life. M2CAB is formulated as an extended-release injectable suspension (XVIR-110) for intramuscular (IM) administration that may achieve ultra-long-acting therapeutic CAB exposures with infrequent administration.

**Results:** Concentration-time profiles are presented in Figure 1 for CAB in rats (Panel A) and for M2CAB and CAB in dogs (Panel B) below. A single IM administration XVIR-110 resulted in low exposures of the prodrug M2CAB but yielded high and persistent exposures of active CAB in both rats and dogs over the period of study to date, 7 and 5 months, respectively. The study is ongoing and based on the projected apparent terminal elimination half-life of CAB, concentrations above the PB-IC_{50} of CAB are expected to remain for more than 12 months. Based upon modelling, these data suggest that XVIR-110 could be dosed once- or twice-yearly in humans. XVIR-110 was well tolerated without evidence for dose-limiting ISRs and demonstrated less pronounced early post-injection microscopic changes, including tissue necrosis, inflammation, and immune cell infiltration vs. commercial cabotegravir at equivalent doses.

**Conclusion:** XVIR-110 demonstrated sustained CAB exposures and a favorable ISR profile in nonclinical species. As such, XVIR-110 may be ideally suited where daily, self-directed, or more frequent IM or SQ may be less desirable or feasible, particularly where inconsistent and/or ad hoc adherence is a risk, such as pre-exposure prophylaxis (PrEP) or in patients with known adherence challenges.

**Effect of Remdesivir on Post-COVID Conditions Among Individuals Hospitalized With COVID-19 by Age**

Mark Berry, Amanda M. Kong, Roger Paredes, Rohan Shah, Gina Brown, Rikisha Gupta, Sohul A. Shuvo, Robert L. Gottlieb, Lourdes Mateu, Mazin Abdelghany, Jason D. Goldman, Anand P. Chokkalingam

Gilead Sciences, Inc, Foster City, CA, USA, Action, Inc, New York, NY, USA, INSAs Institute for AIDS Research, Barcelona, Spain, Baylor University Medical Center, Dallas, TX, USA, Hospital Germans Trias i Pujol, Barcelona, Spain, University of Washington, Seattle, WA, USA

**Background:** Post-COVID conditions (PCC), or long COVID, are part of a persistent, multisystemic syndrome occurring after COVID-19. The effect of the antiviral remdesivir (RDV) on subsequent outcomes associated with PCC is unknown. Of particular interest are RDV’s effects stratified by age, which is a predictor of outcomes in patients hospitalized with COVID-19.

**Methods:** The HealthVerity database of hospital chargemaster data linked to closed claims for >2.5 million US patients was queried for individuals aged ≥12 years hospitalized for ≥2 days with COVID-19 between 5/1/2020 and 9/30/2021. The analysis was stratified by age category (<65 vs ≥65 years of age). Cox proportional hazards models used inverse probability of treatment weighting to calculate hazard ratios (HR) for 16 individual PCC-related symptoms or diagnoses and a composite of any PCC, occurring 90-270 days posthospitalization, in patients hospitalized with COVID-19 receiving RDV versus comparators not receiving RDV. Individuals without >90 days of follow-up still contributed person-time up to their day of censoring.

**Results:** Of 3,661,303 individuals hospitalized for any reason during the study period, 52,006 had acute COVID-19 and met inclusion criteria, of which 33,578 (64.6%) were <65 years of age. In the <65 and ≥65 age groups, respectively, 36.1% and 27.2% received RDV. The most common PCC-related symptom/diagnosis was neuropsychiatric features, with an incident rate per 100 person-years of 58.0 and 52.4 in <65 and ≥65 age groups, respectively. Overall, RDV (vs no RDV) was associated with significantly lower relative hazard of any PCC in both age groups: HR 0.90 (95% confidence interval [CI]: 0.86–0.93) in those <65 years old and HR 0.90 (95% CI: 0.86–0.95) in those ≥65 years old. RDV was associated with lower risk for 6 of 16 individual symptoms/diagnoses in the ≥65 age group (including the same 6 symptoms, as well as thromboembolic disease and headache).

**Conclusion:** RDV was associated with reduced risk of PCC after COVID-19 hospitalization in patients <65 and ≥65 years of age, though more symptoms were impacted, and the effect size tended to be stronger in the younger age group. The majority of patients did not receive RDV, indicating a missed opportunity for treatment of acute COVID-19 and potential prevention of long-term sequelae of infection.
Extended Nirmatrelvir/Ritonavir Treatment Durations for Immunocompromised Patients With COVID-19

Edward Weinstein1, Mary Almas1, Mary Lynn Baniecki1, Elena Tudone2, Simone Antonucci3, Kevin Gregg4, Carolina Garcia-Vidal5, Adrian Camacho5, Wayne Wiseman6, Steven Terr7, Jennifer Hammond1, James Ruskak1
Pfizer, Inc, New York, NY, USA, 1University of Michigan, Ann Arbor, MI, USA, 2Hospital Germans Trias i Pujol, Barcelona, Spain, 3Hospital Clínico de Barcelona, Barcelona, Spain, 4Hospital Universitario Dr. Jose Erodinso Gonzalez, Monterrey, Mexico

Background: Nirmatrelvir/ritonavir (NMV/r) is an FDA-approved treatment for adults with mild to moderate COVID-19 who are at high risk for progression to severe disease. Limited data support dosing recommendations in immunocompromised (IC) patients. This study compared the approved 5-day regimen with 10- and 15-day regimens in IC patients.

Methods: This multinational, randomized, double-blind, phase 2 study enrolled 156 nonhospitalized IC patients ≥12 years of age with symptomatic COVID-19 who tested SARS-CoV-2–positive within 5 days of study entry. Subjects were randomized 1:1:1 to receive 300/100 mg NMV/r twice daily for 5, 10, or 15 days. Nasopharyngeal (NP) swabs for PCR and rapid antigen testing were collected at baseline and on Days 5, 10, 15, 21, 28, 35, and 44. The primary endpoint was proportion of subjects with NP SARS-CoV-2 RNA below the lower limit of quantification (LLOQ; defined as 2.0 log10 copies/mL) from Days 15 through 44.

Results: The primary endpoint was achieved in 62%, 71%, and 66% of subjects in the 5-, 10-, and 15-day treatment groups, respectively. No formal hypothesis testing was performed. The median time to achieving sustained NP SARS-CoV-2 RNA <LLOQ through Day 44 was numerically longer in the 5-day treatment group (15 days) compared with the 10-day (11 days) and 15-day (10 days) treatment groups. An increase in viral RNA following the end of treatment was not observed in nirmatrelvir/r treated pts in all subgroups by baseline symptom severity, serostatus, viral load (VL), time since symptom onset, sex and age; with largest reductions among pts with moderate/severe symptoms at baseline (13 vs 17 d; p=0.0001), seronegative pts (13 vs 17 d; p=0.0001), pts with baseline VL ≥7 log10 copies/mL (14 vs 19 d; p=0.0001), and pts >60 years of age (13 vs 18 d, p=0.0024) (Figure 1). The reduction in median time to sustained symptom resolution was more apparent in the same subgroups: pts with moderate/severe symptoms at baseline (20 vs 23 d; p=0.0101), seronegative pts (19 vs 23 d; p=0.0086), pts with baseline VL ≥7 log10 copies/mL (19 vs 25 d; p=0.0272), and pts >60 years of age (18 vs 22 d, p=0.0386).

Conclusion: Nirmatrelvir/r treatment reduced median times to sustained alleviation and resolution of all targeted COVID-19 symptoms vs placebo significantly overall, and consistently across pt subgroups (by baseline symptom severity, serostatus, VL, time since symptom onset, sex and age) in pts at high risk for progressing to severe disease.

Figure 1. Median time to sustained alleviation of all targeted COVID-19 symptoms through Day 28 by baseline status (mITT1 population)
hospitals achievable through complete compliance with oral antiviral treatment guidelines in high-risk adults.

**Methods:** Using electronic health record data from health systems in Minnesota and Nevada, we identified oral antiviral-eligible SARS-CoV-2 patients between April 2022 and June 2023 as those ≥50 years old, unvaccinated, or with ≥1 high-risk medical condition. Modified Poisson regression was employed to estimate adjusted risk ratios (RRs) for the association between nirmatrelvir/ritonavir (N/r) or molnupiravir (MPV) treatment and hospitalization within ≤14 days after the positive test, stratified by immunocompromised status. Using adjusted RRs and treatment prevalence, we also calculated the preventable fraction of hospitalizations that could have been averted had all eligible patients been treated.

**Results:** Among 3,525 SARS-CoV-2 patients with risk factors for severe COVID-19, 2,223 (63.1%) were ≥50 years old (35.4% ≥65), 829 (23.5%) were unvaccinated, and 2,932 (83.2%) had ≥1 high-risk medical condition. Overall, 1,033 (29.3%) were prescribed an oral antiviral agent (911 N/r; 122 MPV) and 300 (8.5%) were hospitalized. Only 1.9% of treated patients vs. 11.2% of untreated patients were hospitalized (p < 0.001). In adjusted analyses, treatment with any oral antiviral agent (RR = 0.18), with N/r (RR = 0.21), and with MPV (RR = 0.05) were each significantly associated with a lower risk of hospitalization (Figure). The protective association for any antiviral was stronger in immunocompromised (RR = 0.11) vs. immunocompromised (RR = 0.37) patients (interaction, p < 0.001). If all patients had been treated, 76.8% (95% CI: 66.6-85.9) of all hospitalizations could have been averted, including 85.7% (95% CI: 74.5-94.9) of those in immunocompromised and 54.9% (95% CI: 32.4-77.0) of those in immunocompromised patients.

**Conclusion:** Three-quarters of COVID-19 hospitalizations in high-risk patients could have been prevented had all patients received oral antiviral treatment prior to disease progression. This noteworthy finding is attributed to the strongly observed protective effect of treatment coupled with large treatment gaps. Moreover, the diminished protection among immunocompromised patients underscores the importance of close symptom monitoring and consideration of additional therapeutic options such as remdesivir and COVID-19 convalescent plasma.

661 Poled Analysis of Randomized Trials Comparing Drug Efficacy for Early COVID-19 Among Omicron Waves

Valentina Mazzotta¹, Fulvia Mazzaferri¹, Simone Lanini², Massimo Miranda³, Alessandro Cozzi-Lepri¹, Alessia Perissé¹, Alessandro Vergori¹, Alessio Maccarrone¹, Valentina Mazzotta¹, Marco Martini⁴, Andrea Antinori⁵, Fabrizio Maggi⁶, Emanuele Nicastri⁷, Andrea Antinori⁵, for the Early Treatment for COVID-19 Lazio Study Group

¹IRCCS Lazzaro Spallanzani, Rome, Italy, ²University College London, London, United Kingdom, ³University of Rome Tor Vergata, Rome, Italy, ⁴Catholic University of the Sacred Heart, Milan, Italy

**Background:** Although widespread vaccination and lower pathogenicity of the omicron variant had drastically reduced the rate of COVID-19-related hospitalization/death (CovH/D), real-world evidence can help to identify categories still at risk of severe outcomes and inform on the efficacy of different treatments used.

**Methods:** Multicenter cohort of high-risk outpatient pts treated with monoclonal antibodies (mAbs) or antivirals for mild-to-moderate COVID-19 from March 2021 to May 2023 in the Latium Region. The outcome was CovH/D by day 30 from baseline by fitting logistic regression models, including a specific set of potential confounders, for each exposure of interest: age >75 years; vaccination; calendar period (reflecting the main circulating VOC), immunocompromised status. Among pts enrolled in 2022, the difference in risk between interventions (Sotrovimab = SOT; Molnupiravir = MLP; Remdesivir = RDV; tixagavimab/cilgavimab = T/C, nirmatrelvir/ritonavir = N/r) was estimated in emulated parallel trials using a marginal structural model.

**Results:** 12,466 pts enrolled [female 50.2%, median age 70 yrs (IQR 57-80), unvaccinated 21%, immunocompromised 23.2%]. Primary endpoint occurred in 384/12,466 pts, with a day-30 incident risk of 3.08% (95% CI 2.7-3.4%). After controlling for potential confounders, a higher risk was observed for older aged (OR 2.01; 1.64-2.46), unvaccinated (2.30; 1.73-3.05), and immunocompromised (1.41; 1.09-1.82). Using the “Delta period” as a reference, a decreased risk was observed in the Omicron waves. Among the 10,042 pts treated in 2022 (1,919 SOT, 3,733 MLP, 1,405 RDV), failure rate according to intervention varied from 0.87% (0.55-1.32) for N/r, 1.68% (1.2-2.1) for MLP, 3.0% (1.6-5) for T/C, 3.5% (2.7-4.5) for SOT and 5.1% (4.1-6.4) for RDV. Emulation trial for comparison of different treatment options showed higher efficacy of oral antivirals in the prevention of CovH/D compared to mAbs or RDV; no significant differences were observed between the oral antivirals or between mAbs and RDV, respectively.

**Conclusion:** Despite the decreasing risk of CovH/D across the calendar periods, older aged, unvaccinated, and immunocompromised patients remained at the highest risk of developing severe COVID-19. Oral antivirals showed higher efficacy in reducing Cov/H, while no significant differences were observed between them or between mAbs and RDV. These data could help to tailor therapies according to different risk factors and specific contraindications. The figure, table, or graphic for this abstract has been removed.
664 Remdesivir Reduces Mortality in Immunocompromised Patients Hospitalised for COVID-19 During Omicron

Essey Mozaffari1, Aastha Chandak1, Alpesh N. Amin1, Robert L. Gottlieb8, Andre C. Kalil1, Mark Berry1, Alpesh N. Amin, Tobias Welte8, Paul E. Sax1

1Gilead Sciences, Inc, Foster City, CA, USA; 2Cerner, U.S. + Mexico; 3University of California Irvine, Irvine, CA, USA; 4Baylor University Medical Center, Dallas, TX, USA; 5University of Nebraska Medical Center, Omaha, NE, USA; 6University of California Los Angeles, Los Angeles, CA, USA; 7Medizinische Hochschule Hannover, Hannover, Germany; 8Brigham and Women’s Hospital, Boston, MA, USA

Background: Previous research has established the effectiveness of remdesivir (RDV) in reducing mortality among immunocompromised patients hospitalized for COVID-19. In this study, we present data from the Omicron predominant era (Dec’21 – Apr’23) by examining in-hospital all-cause mortality for early RDV initiation vs. not initiating RDV among immunocompromised hospitalized COVID-19 patients.

Methods: Using the PINC AI Healthcare database, adults with immunocompromised conditions (cancer, transplant, hematologic malignancies, immunosuppressive medications, toxic effects of antineoplastics, primary immunodeficiencies, severe combined immunodeficiencies, asplenia, bone marrow failure/aplastic anemia, or HIV) hospitalized with a primary discharge diagnosis of COVID-19 flagged as “present-on-admission” from Dec’21 to Apr’23 were identified. Analyses were stratified by no supplemental oxygen charges (NSOc) and any supplemental oxygen requirements upon admission. Patients initiating RDV in the first 2 days of admission vs. those not initiating RDV during the hospitalization were matched using 1:1 preferential within-hospital propensity matching with replacement. Time to 14- and 28-day in-hospital mortality or discharge to hospice was examined using Cox Proportional Hazards Model.

Results: In the study period, 10,687 RDV-treated patients were matched to 4,989 unique non-RDV patients. Post-matching balance was achieved with 74% being 65+ years, 49% with NSOc, and 51% with any supplemental oxygen charges. Unadjusted mortality rate for RDV patients vs. non-RDV patients was 10.3% vs. 13.7% at 14 days and 15.0% vs. 19.2% at 28 days, respectively. After adjusting for baseline and clinical covariates, RDV showed significantly lower mortality risk compared to non-RDV overall (adjusted hazard ratio [95% CI]: 0.75 [0.68-0.83]) in patients with NSOc (0.72 [0.61-0.85]) and in patients with any supplemental oxygen requirement (0.77 [0.68-0.87]) at 28 days. A similar benefit for RDV vs. non-RDV was observed for 14-day mortality overall (0.73 [0.65-0.82]) in patients with NSOc (0.69 [0.57-0.83]) and in patients with any supplemental oxygen requirement (0.75 [0.65-0.86]) (Figure).

Conclusion: RDV continues demonstrating significant mortality reduction among immunocompromised patients hospitalized with a primary diagnosis of COVID-19 in the more recent Omicron period, irrespective of the supplemental oxygen requirements.

Time to 14- and 28-day mortality in immunocompromised hospitalized patients for COVID-19 by supplemental oxygen requirements (adjusted Cox Proportional Hazards model)

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<td>21,374</td>
<td>0.73 [0.65-0.82]</td>
<td>&lt;0.0001</td>
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<tr>
<td>NSOc</td>
<td>10,442</td>
<td>0.69 [0.57-0.83]</td>
<td>&lt;0.0001</td>
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<tr>
<td>Any Supp. O2</td>
<td>10,932</td>
<td>0.75 [0.66-0.86]</td>
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<td>10,932</td>
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665 Remdesivir + Dexamethasone vs Dexamethasone for the Treatment of COVID-19: Real-World Study in the US

Essey Mozaffari1, Aastha Chandak1, Robert L. Gottlieb8, Chidimma Chima-Melton1, Mark Berry1, Thomas Oppelt1, Jason F. Okulicz1, Alpesh N. Amin1, Andre C. Kalil1, Tobias Welte8, Paul E. Sax1

1Gilead Sciences, Inc, Foster City, CA, USA; 2Cerner, U.S. + Mexico; 3University of California Irvine, Irvine, CA, USA; 4Baylor University Medical Center, Houston, TX, USA; 5University of California Los Angeles, Los Angeles, CA, USA; 6University of California Irvine, Irvine, CA, USA; 7University of Nebraska Medical Center, Omaha, NE, USA; 8University of California Los Angeles, Los Angeles, CA, USA

Background: Dual therapy with remdesivir (RDV) and dexamethasone (DEX) among patients with COVID-19 has demonstrated improved clinical outcomes compared to DEX monotherapy. We evaluated the effectiveness of RDV+DEX vs. DEX monotherapy by applying and comparing two established methods used to balance two inherently different groups due to confounding by indication in observational research.

Methods: Adults hospitalised during the Omicron period (Dec’21–Apr’23) with a primary discharge diagnosis of COVID-19 flagged as “present-on-admission” who initiated RDV+DEX or DEX monotherapy in the first 2 days of hospitalisation (baseline period) were identified in the PINC AI Healthcare database. Patients were categorized by baseline supplemental oxygen requirement: no supplemental oxygen charges (NSOc), low-flow oxygen (LFO), high-flow oxygen/non-invasive ventilation (HFONIV), or invasive mechanical ventilation (IMV)/ECMO. Balanced distribution of underlying confounders in the treatment groups was achieved through 1) 1:1 propensity score matching (PSM) without replacement, which estimates the effectiveness of RDV+DEX by matching patients in the two groups excluding unmatched patients and 2) Inverse probability of treatment weighting (IPTW), which estimates the effectiveness...
of RDV+DEX in the full cohort keeping all eligible patients in the analysis. Cox Proportional Hazards Model was used to assess time to 14- and 28-day mortality.

**Results:** Among 151,215 hospitalized patients for COVID-19, 61,236 (40%) initiated RDV+DEX and 36,489 (24%) DEX monotherapy in the first 2 days. Using PSM, 33,089 RDV+DEX patients were matched to 33,089 DEX monotherapy patients. RDV+DEX had a significantly lower mortality risk compared to DEX monotherapy across all supplemental oxygen requirements at 14 days (HR=0.76 [0.68-0.83]), 28 days (HR=0.71 [0.64-0.79]), and 56 days (HR=0.69 [0.60-0.81]) (Fig). Using IPTW, consistent results were obtained across all supplemental oxygen levels (Fig).

**Conclusion:** The effectiveness of RDV+DEX in reducing mortality compared to DEX monotherapy was confirmed through two well-established methods of addressing confounding by indication bias, thus providing confidence in the observed effectiveness of RDV+DEX therapy. Appropriate methodologies such as the ones applied in this study enables the use of real-world data to complement findings from RCTs.

### Real-Life Experience on the Use of Remdesivir: A Propensity Score Matched Analysis

**Background:** Remdesivir (RDV) was the first FDA-approved medication for COVID-19, with discordant data on efficacy in reducing mortality risk and disease progression. In the context of a dynamic and rapidly changing pandemic landscape, the utilization of real-world evidence is of utmost importance. The objective of this study is to evaluate the impact of RDV on patients who have been admitted to two university referral hospitals in Italy due to COVID-19.

**Methods:** All patients older than 18 years and hospitalized at two different universities (Bari and Palermo) were enrolled in this study. To minimize the effect of potential confounders, we used propensity score matching with one case (remdesivir) and one control that never experienced this kind of intervention during hospitalization. Mortality was the primary outcome of our investigation, and it was recorded using death certificates and/or medical records. Severe COVID-19 was defined as admission to the intensive care unit or mortality.

**Results:** Among 151,215 hospitalized patients for COVID-19, 61,236 (40%) initiated RDV+DEX and 36,489 (24%) DEX monotherapy in the first 2 days. Using PSM, 33,089 RDV+DEX patients were matched to 33,089 DEX monotherapy patients. RDV+DEX had a significantly lower mortality risk compared to DEX monotherapy across all supplemental oxygen requirements at 14 days (HR=0.76 [0.68-0.83]), 28 days (HR=0.71 [0.64-0.79]), and 56 days (HR=0.69 [0.60-0.81]) (Fig). Using IPTW, consistent results were obtained across all supplemental oxygen levels (Fig).

**Conclusion:** The effectiveness of RDV+DEX in reducing mortality compared to DEX monotherapy was confirmed through two well-established methods of addressing confounding by indication bias, thus providing confidence in the observed effectiveness of RDV+DEX therapy. Appropriate methodologies such as the ones applied in this study enables the use of real-world data to complement findings from RCTs.

### Molnupiravir vs Favipiravir: An RCT in Outpatients At-Risk for COVID-19 in Thailand

**Background:** In December 2021, molnupiravir was granted a US FDA’s emergency use authorization for patients with mild-to-moderate COVID-19 at high risk for progression to severe COVID-19 for whom alternative approved or authorized treatment options are not accessible or clinically appropriate. The efficacy of molnupiravir in a vaccinated population predominantly infected with the SARS-CoV-2 Omicron variant is unknown.

**Methods:** In an open-label, parallel-group, multicenter trial in Thailand, outpatients with confirmed SARS-CoV-2 infection for ≤5 days, mild to moderate COVID-19 attributable symptoms/signs for ≤5 days (and ≥7 days before randomization) and ≥1 risk factor for severe COVID-19 were randomly assigned 1:1 to receive oral molnupiravir 800 mg BD for 5 days or oral favipiravir 1800 mg BD on Day 1 then 800 mg BD until Day 5, extended to Day 10 if clinical progression. Phone calls for remote symptom assessment were made on Days 6, 11 (if favipiravir extended), 15 and 29. Participants with worsening symptoms were instructed to return to the hospital. The primary endpoint was pulmonary involvement by Day 29, as evidenced by ≥2 of the following: dyspnea, oxygen saturation <92% or imaging. 465 evaluable participants per arm (930 overall) were needed to have 80% power to detect a difference in the percentage of participants with an endpoint event, assuming 3% in molnupiravir arm and 7% in favipiravir arm (1-sided α=0.024 for final analysis).

**Results:** 977 participants (487 molnupiravir, 490 favipiravir) were enrolled from 8 July 2022 to 19 January 2023. 55% were female and median age was 56 years (interquartile range, 41-64), 98% had received ≥1 dose of COVID-19 vaccine and 83% ≥3 doses. The most common risk factors known to be associated with severity were hypertension (49%) and age ≥60 years (42%). By Day 29, pulmonary involvement occurred in 0% (0/483) in molnupiravir arm versus 1% (5/482) in favipiravir arm (~1.0%); Newcombe 95.2% CI: -2.4% to ~0.0%; p=0.021); all-cause death in 0% (0/483) and <1% (1/482, from COVID-19 pneumonia); COVID-19 related hospitalization in <1% (1/483 and 1% (3/482); and treatment-related adverse event in 1% (5/483) and 1% (4/486). One participant prematurely discontinued molnupiravir on Day 4 due to rash.

**Conclusion:** Rates of pulmonary involvement and of other adverse outcomes were much lower than anticipated in both arms in this population highly vaccinated and mostly infected with Omicron. Pulmonary involvement was less common with molnupiravir than with favipiravir.
668    AGILE CST-8 Phase I Trial of Combined Nirmatrelvir/r and Molnupiravir for Mild-Moderate COVID-19
Sayeh Khoob, Richard J. Fitzgerald, Shazad Ahmad, Chris Edwards, Geoff Saunders, Emma Knox, Calley Middleton, William Greenhalgh, Laura Else, Victoria Shaw, Thomas Fletcher, Helen Reynolds, Gareth Griffiths, for the AGILE CST-8 Study Team
University of Liverpool, Liverpool, United Kingdom, University of Manchester, Manchester, United Kingdom, University of Southampton, Southampton, United Kingdom, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Background: AGILE (NCT04746183) is the UK platform for early-phase evaluation of COVID-19 antivirals. AGILE-CST-8 is a phase I, adaptive, dose escalation study of combination nirmatrelvir/ritonavir plus molnupiravir.

Methods: Adults with a positive lateral flow test, within 5 days of mild-moderate symptoms were randomised (2:1, open-label) to nirmatrelvir/ritonavir plus molnupiravir versus Standard of Care (SoC). Exclusions were oxygen saturation <92%, liver or renal impairment, drug interactions and pregnancy. The primary endpoint was dose-limiting toxicities (DLTs, AEs ≥ grade 3) up to day 11; safety review after each cohort (N=6) guided stepwise dose-reduction of molnupiravir from a starting dose of 800mg bd, reducing to 600mg or 400mg bd if required, with nirmatrelvir/ritonavir maintained at standard doses throughout. A Bayesian dose de-escalation model was used to estimate DLTs. We collected a combined throat/nose swab daily for 5 days, then at day 11 for virology, and measured antiviral concentrations in plasma, saliva, tears and nasal secretions.

Results: Four cohorts totalling 24 participants (16 combination therapy, 8 SoC; 17 female) with median (range) age 35.5 years (20-70), and BMI 27 (20.2-40.2) were enrolled, at a median of 4 days (1-5) from symptom onset. No DLTs or SAEs were observed through to Day 29; all participants randomised to combination therapy received full doses of both drugs apart from one subject withdrawal on day 2 (Bayesian dose-toxicity model shown in Figure). AEs (Grade 1-2) were reported in 14/16 (87.5%, mainly gastrointestinal disorders and altered taste) of combination treatment versus 5/8 (62.5%) SoC participants. At baseline all but one (SoC arm) were SARS-CoV-2 PCR-positive. There was no evidence of differences between participants receiving combination antivirals versus SoC in baseline VL (median 5.2 vs 6.8 log10 copies), or at any point across the first 5 days, or at day 11. The median reduction in VL at Day 5 was 5.0 vs 5.7 log10 copies, with 11/16 (68.8%) of treated vs 5/8 (62.5%) of SoC participants achieving negative PCR. Nirmatrelvir concentrations in tears, nasal secretions, and saliva approximated to 86%, 70% and 18% of plasma.

Conclusion: This first report of combination nirmatrelvir/ritonavir with molnupiravir confirms safety and tolerability at full doses used in adults. The clinical and virological benefits of this combination should be tested in larger phase II studies.

669    Viral and Symptom Rebound After COVID-19: Monoclonal Antibody Therapy in the ACTIV-2 Trial
Kara W. Chew, Brooke McGinley, Carlee Moser, Jonathan Z. Li, Teresa H. Ewing, Justin Ritz, David Margolis, David Weihl, Michael D. Hughes, Eric Drasar, Judith S. Currier, Joseph J. Eron, Davey M. Smith*, for the ACTIV-2/A5401 Study Team
University of California Los Angeles, Los Angeles, CA, USA, Boston University, Boston, MA, USA, Harvard TH Chan School of Public Health, Boston, MA, USA, Harvard Medical School, Boston, MA, USA, VA Wall Cornell Medicine, New York, NY, USA, 3Bri Biosciences, Inc, Durham, NC, USA, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 4Harbor-UCLA Medical Center, Torrance, CA, USA, University of California San Diego, La Jolla, CA, USA

Background: Risk of viral and symptom rebound after monoclonal antibody (mAb) therapy for COVID-19 is unknown.

Methods: Viral and symptom rebound in a randomized placebo-controlled trial of combination mAbs Amubarviamab/Romlusevimab (A/R), which had been shown to reduce hospitalization/death by 79%, were explored. Symptom relapse was defined as first occurrence of any moderate/severe symptom (13 symptoms recorded in a daily 24-h diary) persisting for ≥2 consecutive days, after meeting a 2-days sustained symptom improvement or resolution endpoint. End of relapse was defined as the last day with any moderate/severe symptom after improvement or any mild/moderate/severe symptom after symptom resolution. Viral rebound was defined as anterior nasal SARS-CoV-2 RNA ≥ 10^10 copies/mL at day 7, 14, or 28 that was ≥ 0.5 log10 copies/mL higher than day 3, or at day 14 or 28 that was ≥ 0.5 log10 copies/mL higher than day 7. Wilcoxon rank sum tests compared symptom outcomes by arm, by ranking participants in four ordered categories: 1) improved and never relapsed ordered by symptom duration; 2) improved but relapsed ordered by total time with symptoms; 3) never improved ordered by last available total symptom score (TSS, summing scores for all 13 symptoms on that day), 4) deaths. Joint modeling of time to improvement and to relapse assessed the risk of relapse among those with improvement or resolution, adjusting for day 0 TSS. Proportion of participants with viral rebound was compared between arms using Fisher’s Exact test.

Results: 784 participants were included. Median age was 49 years; 52% female (>99% censored); 72% White and 49% Latino/Hispanic, 75% A/R and 73% placebo participants achieved symptom improvement; 5% in each arm had symptom relapse (Table). 1 placebo participant with relapse was hospitalized. Fewer participants met symptom resolution (64% A/R, 60% placebo) and relapse after resolution (3% and 2%, respectively, none subsequently hospitalized) (Table). Time to symptom relapse did not differ between arms among those who achieved improvement or resolution (Table). Viral rebound occurred in 4% in each arm (p=0.99). 3 participants with viral rebound were hospitalized, all in the placebo arm. <1% of each arm experienced both symptom and viral rebound.

Conclusion: This randomized trial found no significant differences in symptom experiences or viral rebound between mAb- vs placebo-treated participants. With or without treatment, rebound rates were low following sustained symptom improvement or resolution.

### Table

<table>
<thead>
<tr>
<th>Symptom Category</th>
<th>A/R (n=397)</th>
<th>Placebo (n=387)</th>
<th>p-value</th>
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<tr>
<td>Improvement</td>
<td>34/11 (86%)</td>
<td>17/21 (81%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Relapse</td>
<td>13/28 (46%)</td>
<td>16/32 (50%)</td>
<td>0.49***</td>
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</table>

Table. Symptom relapse following 2 days sustained symptom improvement or 2 days sustained symptom resolution, by treatment arm. *p-value corresponded to McNemar rank sum-comparing ordered data across 4 categories. **p-value improvement/resolution to relapse among those who relapsed. ***p-value Chi-Square.

670    Risk of SARS-CoV-2 Infection in Patients With Hematologic Diseases Receiving Tixagevimab/Cilgavimab
Alessandra Vergori, Alessandro Cozzoli-Lepri, Marta Chiucciarelli, Valentina Mazzotta, Elisabetta Metafuni, Giulia Matsali, Valentina Siciliano, Jessica Paulicelli, Eleonora Alma, Agostina Siniscalchi, Elisabetta Abbruzzese, Simona Sica, Massimo Fantoni, Andrea Antinori, Antonella Cingolani
Lazzaro Spallanzani National Institute for Infectious Diseases, Rome, Italy, University College London, London, United Kingdom, Catholic University of the Sacred Heart, Rome, Italy, San Filippo Neri Hospital, Rome, Italy, Sant’Eugenio Hospital, Rome, Italy

Background: Despite in vitro data showing poor neutralizing activity, clinical efficacy of tixagevimab/cilgavimab (T/C) as pre-exposure prophylaxis (PrEP) in patients (pts) with hematologic disease (HD) against the newest omicron sub-variants of SARS-CoV-2 was rarely investigated and long-term incidence data are lacking.
**Methods:** Observational study of pts with HD who received 300 mg of T/C given intramuscularly as PrEP over MAR22-AUG23. Demographic and clinical characteristics were collected; BTIs were defined as a confirmed diagnosis by RT-PCR and clinical picture. The incidence of BTI (95%CI) was calculated using the Kaplan-Meier method. Factors associated with the risk of BTI were evaluated using a Cox regression model with fixed covariates after controlling for age, sex, type of HD and other comorbidities. The incidence of BTI was also calculated according to the circulating variant (VoC) as number of BTI per 100 PYFU. A Poisson regression adjusted for the same potential confounders was used to estimate relative rates of BTI by current VoC.

**Results:** N=501 pts: 68% initiated T/C PrEP when BA.5 was the most prevalent, followed by XBB/EG, BA.2 and BA.1 (21%, 7% and 3%, respectively); 46% female, median age 64 years (IQR 55, 73) and a median follow-up post treatment of 162 (92-297) days. HD were non-Hodgkin Lymphoma (NHL) 66%, Multiple Myeloma (MM) 12%, Chronic Lymphocytic Leukemia (CLL) 14%, Hodgkin Lymphoma (HL) 8%; 3% received CAR-T and 9% bone marrow transplantation. 87% received 3-4 vaccine doses 60% had other comorbidities. Overall, the 1-year incidence estimate of BTIs was 21% (16.5-26.8%), with a cumulative risk which was higher than the longer the time since PrEP. A greater risk of incident BTIs was observed when BA.5 and XBB/EG sub-lineages circulated as prevalent in Lazio region (aRR 5.08 [2.18, 11.83]; p<0.001 and 3.77 [1.48, 9.60]; p=0.005, compared to BA.1, respectively). The 6-month incidence of BTIs was higher for MM [25%-2-47%] than that seen for other HDs. Severe COVID-19 occurred in 6 pts; 4 deaths due to COVID-19 were reported.

**Conclusion:** One-year incidence of BTIs after T/C PrEP was <25%, although increasing over time, possibly due to diminished neutralizing activity. The risk appears to be higher when more recent omicron sub-lineages were circulating suggesting a lack of in vitro neutralization due to viral escape. The figure, table, or graphic for this abstract has been removed.

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**671 Immunogenicity of Tixagevimab/Cilgavimab for COVID-19 in Immunocompromised Children and Adolescents**

Jassada Buaboonnag1, Supapat Rungrma1,1, Nuntawan Piyaphanee2, Sirirat Charuvanij2, Onsiri Pitisuttithum2, Katherine Copeland3, Chatkamol Pheerapanyawaran1, Suvonmil Niyomnaitham2, Kulkanya Chokephaibulkit2

**Background:** COVID-19 vaccination prevents severe manifestations, hospitalization, and mortality in healthy patients. Immunocompromised populations have a higher risk of complications, but poorer responses to vaccination. Long-acting monoclonal antibodies Tixagevimab/Cilgavimab effectively prevented COVID-19 and improved clinical outcomes when administered early after infection. They were approved for emergency use in ≥40 kg and ≥12 years old immunocompromised patients. Few studied its use in young children. This trial assessed the immunogenicity and safety of an alternative dose in immunocompromised 20-<40 kg children and adolescents.

**Methods:** 6–18-year-old immunocompromised patients were enrolled in this single-center, prospective, open-labelled, randomized clinical trial. Participants were divided into two groups by body weight: 150-mg-Tixagevimab/150-mg-Cilgavimab (≥40 kg) and 300-mg-Tixagevimab/300-mg-Cilgavimab (≤40 kg). Anti-SARS-CoV-2 receptor binding domain IgG (anti-RBD IgG) and pseudovirus neutralizing antibody (NAB) titers were measured as primary outcomes 4, 12, and 24 weeks after administration. Immunogenicity data at 4 weeks was compared with reference data from 5-11-year-old healthy children 2 weeks after a third dose of 10-µg-BNT162b2. Adverse events were closely monitored by telephone calls up to 7 days after administration.

**Results:** Of 59 enrolled participants, 49.2% were female, with a median (IQR) age of 12 (9, 15) years. Fourteen children (24%) had malignancies. Four weeks after administration, similarly high levels of anti-RBD IgG geometric mean concentrations were observed for both arms (6,871 vs 7,486 BAU/mL in 20-40 kg, respectively; P = 0.446), and significantly higher levels than the reference (3,842 BAU/mL two weeks after three doses of BNT162b2; P = 0.001). NAB geometric mean titers (GMTs) for the ancestral Wuhan strain in both arms were similarly high (16,363 vs 17,675 BAU/mL in 20-40 kg, respectively; P = 0.468) and significantly higher than the reference (836 BAU/mL; P < 0.001). NAB GMTs for Omicron BA.4/5 in both arms were on par with the reference. Most adverse events were mild and well-tolerated.

**Conclusion:** Half dose Tixagevimab/Cilgavimab in 20-<40 kg children and adolescents generated equivalent and significantly higher antibodies than ≥40 kg and healthy children with three vaccinations, respectively. This supports further study of next generation long-acting monoclonal antibodies.

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**672 Sotrovimab Lacks Efficacy in Treatment of Syrian Golden Hamsters Infected With SARS-CoV-2 BQ.1.1**

Lee Tatham, Joanne Sharp, Megan Neary, Joanne Herriott, Edyta Kijak, Eduardo Gallardo-Toledo, Helen Cox, Chloe Bramwell, Anthony Valentijn, Usman Arshad, Henry Pertinex, Paul Curley, Rajith Rajoli, James P. Stewart, Andrew Owen

University of Liverpool, Liverpool, United Kingdom

**Background:** Monoclonal antibodies (mAbs) demonstrate diminished neutralisation of Omicron sub-lineages such that continued use of sotrovimab (SOT) is not supported by current pharmacokinetic-pharmacodynamic (PK-PD) understanding. However, SOT is still used in immunocompromised patients in some geographies. Published preclinical studies have shown virological efficacy in prophylaxis for BQ.1.1 but no data are available in treatment. This study investigated the virological efficacy of SOT in healthy and immunocompromised male hamsters infected with Delta or BQ.1.1 variants using experimental designs reflective of prophylaxis and treatment.

**Methods:** Intramuscular SOT (14 mg/kg) was administered 24h before or after intranasal inoculation with 10^4 PFU Delta or BQ.1.1. Control groups were dosed IM with vehicle 24h after inoculation. A subset of hamsters were immunosuppressed using 100 mg/kg IP-administered cyclophosphamide. Hamsters were sacrificed at 3-dpi and viral replication was quantified using qPCR to measure total (N-gene) viral RNA. Data were normalised to 18S for quantitation. Terminal blood samples were taken and ELISAs were used for SOT plasma quantification.

**Results:** Reductions in pulmonary viral RNA were observed in prophylaxis and treatment for Delta in healthy and immunocompromised animals (Table 1). Smaller and statistically insignificant reductions were observed for BQ.1.1 for prophylaxis and treatment, in healthy and immunocompromised animals. Reduced pulmonary viral RNA was evident in untreated BQ.1.1 inoculated, compared to untreated Delta inoculated, hamsters in healthy and immunocompromised groups (log_10: -3.38; P=-0.007, log_10: -1.85; P=0.0001, respectively). SOT plasma concentrations were comparable across Delta (44.13 ± SD 5.40 µg/mL) and BQ.1.1 (47.06 ± SD 4.44 µg/mL) inoculated groups and consistent with values reported for prior hamster studies.

**Conclusion:** Consistent with PK-PD understanding from RCTs, SOT did not exert virological efficacy in the treatment of immunocompromised hamsters infected with BQ.1.1. SOT did not block infection of Delta or BQ.1.1 when given in prophylaxis. The relevance of changes in viral RNA at high SOT concentrations, early in the profile, to longer-term prophylaxis are unclear. Analysis of pulmonary viral RNA and compartmental SOT concentrations across an extended experimental design is warranted. Caution should be taken when interpreting preclinical experimental designs that may not be reflective of the clinical use case.

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**Table: Mean log_{10} fold changes in SARS-CoV-2 RNA, relative to vehicle control groups, in hamster lung samples at 3-dpi following inoculation with 10^4 PFU Delta or BQ.1.1 variants. Statistical comparisons using unpaired two-tailed t-test.

<table>
<thead>
<tr>
<th>SARS-CoV-2 variant</th>
<th>Delta</th>
<th>BQ.1.1</th>
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</thead>
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<tr>
<td>Healthy</td>
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<td></td>
</tr>
<tr>
<td>Immunocompromised</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOT</td>
<td>log_{10} [SD] 4.44 µg/mL</td>
<td>log_{10} [SD] 4.44 µg/mL</td>
</tr>
<tr>
<td>Reference</td>
<td>3.67 ± 0.30</td>
<td>3.67 ± 0.30</td>
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First-in-Human Phase I Trial of an Adjuvanted SARS-CoV-2 Spike Ferritin Nanoparticle Vaccine

Natalie Collins1, Brittany Ober Shepherd2, Paul T. Scott3, Melanie McCauley1, Jack Hutter1, Christine Lee1, Velsele Guzman1, Adrian McDermott1, Stella Peel1, M. Gordon Joyce1, Merlin L. Robb2, Nelson L. Michaeli3, Sandhya Vasani4, Kayvon Modjarrad1, for the SpFN/ALFQ Study Group

1Walter Reed Army Institute of Research, Silver Spring, MD, USA, 2Henry M Jackson Foundation, Bethesda, MD, USA, 3Sanofi, Marcy l’Etoile, France

Background: Next-generation coronavirus (CoV) vaccines must confer broader protection against a range of SARS-CoV-2 variants as well as novel species that may cross over from zoonotic reservoirs in the future. The SARS-CoV-2 recombinant spike ferritin nanoparticle (SpFN) vaccine co-formulated with Army Liposomal Formulation (ALFQ) adjuvant containing monophosphoryl lipid A and QS-21 (SpFN/ALFQ) was previously shown to be immunogenic and protective in challenge experiments in rodents and non-human primates against SARS-CoV-2 and variants of concern (Voc). This study reports the first-in-human randomized, double-blind, placebo-controlled clinical trial of SpFN/ALFQ.

Methods: Healthy, SARS-CoV-2 seronegative and unvaccinated adults aged 18-55 years, majority male, were randomly assigned to receive either 25μg or 50μg of SpFN/ALFQ or saline placebo intramuscularly at days 1 and 29, with an optional open-label third vaccination at day 181. Local and systemic reactogenicity, adverse events and humoral immunity were quantified and analyzed by Chopper-Pearson. Binding and neutralizing antibody responses against multiple CoV were quantified. To further test breadth of cross-protection, IgG antibodies from vaccinees were infused into Syrian Golden hamsters (SGH) prior to SARS-CoV-1 Urbani challenge. Lung tissue was recovered at days 3 and 6 post challenge. Virus detected by quantitative RT-PCR was analyzed by two-way ANOVA, as well as immunohistochemistry staining.

Results: Of the 29 participants enrolled, all received 2 doses and a subset who did not receive EUA vaccines received a third vaccination. Local and systemic reactogenicity was mild to moderate, and no participants experienced any adverse events of special interest. Binding and neutralizing antibody responses peaked at day 43. Neutralizing antibody titers against Omicron strains were detectable after two immunizations and peaked after the subset of volunteers who received a third immunization and were present at day 361. Passive IgG transferred from vaccinated volunteers into hamsters-controlled replication of SARS-CoV-2 post challenge.

Conclusion: Individuals vaccinated with SpFN/ALFQ mounted neutralizing antibody responses against multiple clade 1 sarbecoviruses. The results of this first-in-human clinical trial represents a platform upon which to build future sarbecovirus vaccine development.

ACTG 5381: Virologic and Resistance Outcomes After Switch to TLD for Failing 1st- or 2nd-Line ART

Carole L. Wallis1, Caitlyn McCarthy1, Catherine Godfrey2, Sarita Shah3, Gissy M. Kityo4, Urvi M. Parikh5, Gary Maartens6, Isaac Tsuikutns7, Fatma F. Some8, Samuel Pierre9, Yvetot Joseph9, Charles W. Flexner10, Michael D. Hughes11, John W. Mellors11, for the ACTG 5381 Team

1BioAnalytical Research Corporation South Africa, Johannesburg, South Africa, 2Haniham TH Chan School of Public Health, Boston, MA, USA, 3US Department of State, Washington, DC, USA, 4Emory University, Atlanta, GA, USA, 5Joint Clinical Research Center, Kampala, Uganda, 6University of Pittsburgh, Pittsburgh, PA, USA, 7University of Cape Town, Cape Town, South Africa, 8Kenya Medical Research Institute, Nairobi, Kenya, 9Mas University, Eldoret, Kenya, 10SHESIKO, Port-au-Prince, Haiti, 11The Johns Hopkins University, Baltimore, MD, USA

Background: Most countries recommend tenofovir-lamivudine-dolutegravir (TLD) for individuals starting antiretroviral therapy (ART) or switching from suppressive 1st-line NNRTI- or 2nd-line PI-based ART but country guidelines have been more variable about using TLD for those with unsuppressed (plasma HIV-1 RNA>1000 c/ml) viral load (VL) ART. We report virologic and resistance outcomes for unsuppressed individuals switching to TLD in the AS381 prospective cohort study.

Methods: Participants were adults or adolescents aged >10y with VL>1000 c/ml switching from 1st-line NNRTI-based (Cohort 1) or 2nd-line PI-based (Cohort 2) ART. Primary endpoints were proportion with VL≤1000 c/ml at 6 months (m) after switch among those still on TLD and proportion with new DTG resistance mutations (baseline and 6m samples were sequenced for those with VL>1000 c/ml at 6m). A case-control study (unsuppressed vs suppressed) evaluated tenofovir diphosphate (TFV-DP) concentrations in dried blood spots.

Results: From Dec2019 to Sep2022, 44 participants were enrolled into Cohort 1 (77% female, median age 33y) and 173 were enrolled into Cohort 2 (57% female, median age 41y) at 13 sites in Haiti and Africa. In Cohort 1, median VL was 4.0 log10 c/ml, CD4 count was 306 cells/mm3, time on ART was 5.5y. In Cohort 2, median VL was 4.2 log10 c/ml, CD4 count was 262 cells/mm3, time on ART was 5.4y. Of 42 participants in Cohort 1 on TLD, 81% had VL≤1000 c/ml at 6 months (m) after switch among those still on TLD and proportion with new DTG resistance mutations (baseline and 6m samples were sequenced for those with VL>1000 c/ml at 6m). A case-control study (unsuppressed vs suppressed) evaluated tenofovir diphosphate (TFV-DP) concentrations in dried blood spots.

Results: There was no difference in the proportion of participants with related Grade 2 or higher AEs between groups. The most frequent AEs were mild nasal irritation and congestion; two participants in the Q-GRFT arm withdrew due to nasal congestion. After adjusting for the dilution factor, Q-GRFT was detected at 50-200x the IC50 in NP and nares swabs at 1 hour and at 1-2x the IC50 at 24 hours after use (Table); results did not differ by sex. ADA was detectable in low levels in plasma in 46% of participants; ADA’s impact on antiviral activity is being assessed. No Q-GRFT was detected in plasma samples (lower limit of quantitation: 3 ng/mL). Acceptability of the nasal spray (somewhat or highly acceptable) was similar between the two groups (87% Q-GRFT vs. 100% placebo, P=0.28).

Conclusion: The Q-GRFT nasal spray administered for a total of 14 doses was well tolerated and demonstrated persistence up to 24 hours in clinically important anatomic sites without systemic absorption. This nasal spray could address the need for an on-demand product for prevention of SARS-CoV-2.
emergence of DTG mutations and lower TFV-DP concentrations in unsuppressed vs suppressed suggest that incomplete adherence to TLD was the major mechanism for failure to suppress viremia.

### Table: Virucologic suppression Rates Over Time (compared to baseline adherence levels)

<table>
<thead>
<tr>
<th>Cohort 1</th>
<th>Cohort 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>2 months</td>
</tr>
<tr>
<td>90%</td>
<td>85%</td>
</tr>
<tr>
<td>80%</td>
<td>75%</td>
</tr>
<tr>
<td>70%</td>
<td>65%</td>
</tr>
</tbody>
</table>

### Methods:

Methods: We present proportions of patients with viremia (VL > 400 copies/mL) 2 years after switching to DTG-based first-line ART between Nov 2019 and Dec 2020, by country and VL at switch. We calculated relative risks (RR) of viremia at 2 years, with exact 95% confidence intervals (CI). We also report major integrase drug resistance mutations (DRMs) detected in 2-year samples among participants with VL > 1000 copies/mL.

### Results:

Results: Of PLHIV switched during the study period, 142/1458 (9.7%) in Malawi and 1410/1417 (99.5%) in Zambia had a viral load measurement available at switch and were eligible. Most participants were women; 1409 (91%) in Malawi and 1169 (83%) in Zambia; median time on ART was 6.1 years. Seventy-seven PLHIV were viremic at baseline in Malawi (5.4%), compared to 42 (3.0%) in Zambia. In Malawi, 1149/1422 (81%) participants had a 2-year VL; the corresponding percentages were 1059/1169 (90%) in Zambia. In Malawi, 1149/1422 (81%) participants had a 2-year VL; the corresponding percentages were 1059/1169 (90%) in Zambia. In Malawi, 777/1422 (5.4%) were viremic at baseline, compared to 22 (1.7%) in Zambia. In Malawi, 777/1422 (5.4%) were viremic at baseline, compared to 22 (1.7%) in Zambia.

### Conclusion:

Conclusion: Viremia was uncommon two years after the programmatic switch to DTG-based first-line ART, and only two cases of emergent DTG drug resistance were detected. Still, PLHIV switching to DTG with viremia had a substantially higher risk of viremia at 2 years than PLHIV with viral suppression at switch. The Zambian policy of only switching virologically suppressed patients may have reduced the risk of developing viremia and virological failure on DTG.

### Table: Virucologic outcomes of PLHIV at 2 years after programmatic switching to Dolutegravir (DTG)-based first-line ART, in two ART programs in Malawi and Zambia.

<table>
<thead>
<tr>
<th>Viral load at switch</th>
<th>Viral load at 2 years</th>
<th>Viral suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malawi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppressed</td>
<td>905</td>
<td>39 (4.3%)</td>
</tr>
<tr>
<td>Viremic</td>
<td>39</td>
<td>15 (3.8%)</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>7.8 (4.2 to 13.4)</td>
<td>54</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.005</td>
<td></td>
</tr>
</tbody>
</table>

| Zambia               |                       |                   |
| Suppressed           | 1189                  | 20 (1.7%)         |
| Viremic              | 20                    | 2 (0.3%)          |
| Relative risk (95% CI) | 3.1 (0.36 to 12.0) | 0.3               |

677 DTG Resistance in Patients with Previous ARV Experience and Viremia in Kenya Receiving DTG-Based ART

### Background:

Background: Kenya began rolling out DTG-based ART in late 2017 as both first- and second-line treatment for adults and children living with HIV (PLHIV). HIV drug resistance (HIVDR) data is limited in PLHIV failing DTG-based ART. We used the cyclical- acquired HIVDR (CADRE) methodology to assess the frequency of HIVDR mutations in PLHIV with detectable viremia on DTG-based ART between January and March 2023 in Kenya.

### Methods:

Methods: The National AIDS and STI control program (NASCoP) collected remnant plasma from HIV viral load testing between January and March 2023 from PLHIV in 15 high/moderate HIV prevalence counties receiving DTG-based ART through the Kenya HIV Care and Treatment Program. We received 191 samples from PLHIV of the expected CADRE-calculated sample size of 622. Plasma was genotyped using thermo-fisher Sanger pro/RT/int sequencing on samples >200 cp/mL and mutations were identified using Stanford HIVdb v9.5. Clinical and demographic data on treatment history and duration was obtained from viral load request forms.

### Results:

Results: Samples were available for 138 of 332 (42%) adults, median age 36 (IQR 24-45) years, and 52 of 290 (18%) children (median age 15 [IQR 12-16] years) with 1 of unknown age. 146 of 191 total (76%) received DTG as 2nd-line, with 141 on NNRTI-based ART and 5 on PI-based ART for a median of 5 [IQR 2-8] years before switching to DTG-based ART; the remaining PLHIV (45 of 191; 24%) received DTG as 1st-line. 56 of 191 (29%) had HIV-1 VL >200 cp/mL, 44 of 56 (79%) were successfully genotyped. 7 of 32 (22%) ART-experienced PLHIV had INSTI resistance with E138K alone or with up to three additional major INSTI mutations including T66I, G140A, S147G, Q148KNR, and/or N155H. All 7 also had multiple NNRTI and NRTI DRMs including 5 with thymidine analogue mutations. 1 of 12 (8%) PLHIV on 1st-line ART had VL >200 cp/mL after 17 months on ART and the INSTI DRM R26K with only K70Q and M184V in RT (Table).

### Conclusion:

Conclusion: The frequency of INSTI HIVDR was high (22%) in ART-experienced PLHIV on DTG-based ART used as 2nd-line ART and lower (8%) in ART-naïve adults using DTG for 1st-line ART, suggesting the risk of failing DTG may be higher in a background of pre-existing mutations to NNRTIs in the regimen. Our data emphasizes the importance of HIVDR monitoring in PLHIV on DTG-based ART given the anticipated introduction of long-acting cabotegravir for HIV prevention and the potential for transmission of INSTI-resistant virus from those on failing DTG-based ART to cabotegravir PEP recipients.

678 Emerging Drug Resistance in Lesotho

### Background:

Background: Since 2018, the World Health Organization has recommended DTG-based antiretroviral therapy (ART) as the preferred regimen for most people with HIV. Most African countries have thus shifted from non-nucleoside transcriptase inhibitor (NNRTI)- to DTG-based ART, often without reviewing prior viral suppression and without access to resistance testing in case of viremia. National programs report higher rates of viral suppression since changing to DTG, including among people with unsuppressed viremia before the change. To date, few DTG resistance-associated mutations (RAMs) have been reported from African routine care cohorts.

### Methods:

Methods: This study aims to assess emerging DTG RAMs among participants of the Viral Load Cohort North-East Lesotho (VICOLE). Eligibility criteria

| HIVDR mutations(INSTI/NNRTI) among PLHIV on DTG-based ART. |
|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                       | Treatment interruption| ART-naïve adults | ART-experienced adults | INSTI/NNRTI mutations |
| 1030                  | Yes                   | 43  | 169  | 169  |
| 7226                  | No                    | 57  | 169  | 169  |
| 9365                  | Yes                   | 40  | 169  | 169  |
| 2516                  | No                    | 49  | 169  | 169  |
| 5152                  | Yes                   | 21  | 169  | 169  |
| 88925                 | No                    | 22  | 169  | 169  |
| 2012                  | Yes                   | 19  | 169  | 169  |

### Table: Virucologic outcomes of PLHIV at 2 years after programmatic switching to DTG-based ART, in two ART programs in Malawi and Zambia.
were i) written informed consent, ii) having changed from NNRTI-based to DTG-based ART, and iii) substantially having ≥2 viral loads ≥50 copies/mL (c/mL), including at least one available sample ≥500 c/mL and taken ≥18 months after changing to DTG (data closure: April 20, 2023). For each participant, the last-available sample with a viral load ≥500 c/mL, as well as previous samples if DTG RAMs were detected, were analysed near full-length HIV sequencing for genotypic resistance testing (GRT). RAMs were interpreted using the Stanford HIVdb (9.5.0).

**Results:** Among 14,881 VICONEL participants who had changed to DTG ≥18 months before data closure, 75 (0.5%) fulfilled the inclusion criteria (median age at change 27 years, 57% female). GRT were available for 54/75 (72%). Among these 54 participants (median age at change 24 years, 54% female), high-level and low-level resistance to DTG was detected in five and one, respectively. None of these six had a documented viral load <50 c/mL <6 months before changing to DTG (Figure). DTG RAMs detected were HS1Y (participant 6 in Figure), E2Q9 (6), G118R (1,2,3,5), E138A (3), E138K (1,2,3,5), N155H (4), and R263K (1,4,5). GRT data from before the change to DTG were available for three of these six participants (participants 1, 3, 6 in Figure): in all cases, RAMs against the nucleoside reverse transcriptase inhibitor backbone but no RAMs in the integrase region were recorded before change.

**Conclusion:** Among participants who changed from NNRTI- to DTG-based ART with sustained viremia ≥18 months after change, DTG resistance was more frequent than expected, highlighting the need for national programs to ensure close follow-up with access to GRT for this specific subgroup.

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**679 Resistance to Second-Generation INSTIs in Mexican PLWH: Emergence of the R263K Mutant**

Jannette A. Juárez-González, Emilio Ivan Sanchez Cruz, Luis A. Angulomedina, Roberto A. Rodríguez-Díaz, Elsa Y. Vidal-Laurencio, Sofía Sierra Vásquez, Luis Enrique Soto Ramírez

**Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico**

**Background:** Clinical trials and real-life experience have shown limited resistance development after use of second generation INSTIs, specially in first line. One of the mutants found in this scenario is the R263K, a nonpolymorphic mutation that alone reduces DTG, BIC, and CAB susceptibility about 2-fold. This mutation has been described in first line failure to second generation INSTIs.

**Methods:** We analyzed all the samples submitted for integrase resistance genotype (Abbott ViroSeq HIV-1 Genotyping System) to our reference laboratory from October 2021 to September 2022. Our lab that performs all the resistance tests from 25 states in México, centers that care for about 2/3 of the cases in the country. In all cases the test was ordered to detect integrase resistance.

**Results:** Ninety-four samples were submitted for integrase resistant tests to our referral laboratory; 77(72%) have RAMs to INSTIs, but only 19 (18%) had primary resistant mutants in 12 of them accompanied by secondary mutations. The most common mutants detected were the combination of Q148H plus G140S in eight cases, all failing to DTG BID after previous failure to RAL; followed by mutation R263K present in 7 cases of them detected in 2023 tests. R263K was detected in 2 cases failing to DTG and 3 to Bictegravir/TAF/emtricitabine (BIC/TAF/FTC) used as a first line treatment. All cases failing to BIC/TAF/FTC had R263K together with M50I, and were failing for at least one year, always with viremias lower than 5,000 copies/mL.

**Conclusion:** Despite the widespread use in México of second generation INSTIs, especially after 2019 were BIC/TAF was used as first line treatment in close to 90% of starting cases; the number of failures is limited. Only 18% of the tests submitted had primary mutations in the integrate gene, mostly in failures after RAL use. The 3 cases of resistance to BIC/TAF/FTC in first line showed a rare mutant combination associated to low viral fitness, and are probably related to bad adherence.
681 Phenotypic Characterization of Replication-Impaired Lenacapavir-Resistant HIV Clinical Isolates
Sally Demerdjian, Vidula Naik, Nicolas Margot, Brie Falkard, Christian Callebaut
Gilead Sciences, Inc, Foster City, CA, USA

Background: Lenacapavir (LEN) is a potent, first-in-class long-acting HIV capsid (CA) inhibitor, approved by the FDA as a twice-yearly treatment option for people living with multi-drug resistant HIV. Out of 258 people with HIV (PWH) who received LEN in clinical studies, CA mutations were observed in 14 participants (M66I, Q67H/K/N, K70R/H/N/R/S, N74D/H, A105T/S, and T107A/C/N/S). Phenotypic analyses of these mutants were successful in a single cycle (SC) assay; however, in the majority of capsid mutants, replication was impaired and too low for phenotyping by the multicycle (MC) MT-2 cytopathic assay. These mutants also had low replication capacity (RC) in the SC PhenoSense Gag-Pro assay (Monogram Biosciences). Here, we have developed and optimized a novel MC phenotyping assay using a Rev-dependent HIV reporter-controlled cell line, Rev-CEM-Luc/GFP (RevLucGFP), to characterize CA mutants with low infectivity.

Methods: The HIV Gag-Protease fragments from plasma samples with CA resistance mutations (CAPELLA and CALIBRATE studies, n=21) and associated site-directed mutants (SDMs, n=15) were cloned into pXXL1A HIV molecular clone followed by transfection into 293T cells. Replicative viral supernatants were evaluated in 2 different MC infection formats; MT-2 and RevLucGFP cell lines, with readouts of cell viability and viral replication, respectively. Patient isolates were also evaluated in the PhenoSense Gag-Pro assay. Outcomes for antiviral assays included fold change (FC) in LEN susceptibility and RC (Gag-Pro assay).

Results: We successfully phenotyped 11 mutants in RevLucGFP cells that were non-infectious in MT-2 assays, including clinical isolates containing M66I in various genetic contexts and combinations of LEN resistance associated mutations (RAMs) with FC ranging from 43.5 to >1000. Antiviral activity and susceptibility in the RevLucGFP MC assay aligned with the previously observed data in the SC PhenoSense Gag-Pro assay and MT-2 cells. Additionally, we observed that SDMs generated in a clinical isolate background containing common polymorphisms have a greater ability to be phenotyped, as compared to SDMs generated in WT lab isolate background. All CA mutants with resistance to LEN remained sensitive to other main HIV drug classes.

Conclusion: Using a sensitive HIV-dependent reporter-based system, we evaluated phenotypic susceptibility of several viruses with low RC (0.6-24% of WT) that were previously uncharacterized, expanding our understanding of LEN resistance and interactions between CA RAMs.

682 Rapid Selection of HIV-2 Capsid Mutations After Failure of a Lenacapavir-Containing Regimen
Mélanie Bertine1, Gilles Peytavin1, Thiibault Saint-Joannis1, Antoine Bachelder1, Pierre de Truchis1, Sylvie Lariven1, Philippe Morlat2, Cécile Poulanger1, Naomi Sayre1, Roland Tubiana1, Nadia Valler1, Charlotte Charpentier1, Diane Descamps2, Jade Ghosn2, Antoine Bachelard3, Coralie Pallier3, Charlotte Charpentier4, Brigitte Montes4, Sabine Yerly5, Roland1, Anne de6, Diane Descamps6, Philippe Morlat6, Marie-Laure Chaix6, Charlotte Charpentier4, for the French ANRS MIE Resistance Study Group, Vincent Calvez6, Magali Bouvier-Alias7, Gilles Peytavin8

Background: Drug resistance is a major hurdle in the treatment of people living with HIV-2 (PLWH-2). The limited number of therapeutic options combined with the rapid selection of mutations can lead to therapeutic dead ends. Lenacapavir (LEN) is the first capsid inhibitor, with in vitro activity against both HIV-1 and HIV-2. It has been approved for PLWH-1 with multi-drug resistant viruses but its genetic barrier is low and several drug-resistance associated mutations (DRAMs) have been reported.

Methods: French PLWH-2 with multi-drug resistant viruses had access to LEN through a compassionate use program. LEN was initiated along with an optimized background regimen (OBR). Plasma viral loads were measured throughout follow-up, and capsid genotyping was performed at time of virological failure using an in-house PCR assay, followed by high-throughput sequencing. Capsid sequences were compared with viral sequences obtained prior to LEN initiation to identify potential DRAMs.

Results: A total of 8 PLWH-2 (4 female) initiated a treatment with LEN and an OBR. The genotypic susceptibility score (GSS) of the OBR was equal to, or below, two for all patients. Plasma viral loads at time of initiation were detectable in 6/8 patients (median: 3,830 copies/mL, range: 665-60,450). Three additional PLWH-2 achieved virological suppression within two to three months after initiating LEN + OBR. Capsid genotyping was performed on the first positive plasma viral load (median pVL: 1,600 copies/mL, range: 260-7,210). Capsid mutations were observed in six PLWH-2: five N73D mutations and one double mutation (Q66H-R69K) (Table). In addition to the N73D mutation, an A76V mutation emerged in the viruses of two patients at 11 and 17 months after initiation of LEN. Two patients (#7 and #8) had persistently high plasma viral loads but their viruses did not select any capsid mutations. Of note, the patient with the highest GSS (#4) had adherence issues, resulting in a functional monotherapy of LEN for several weeks.

Conclusion: We report the rapid selection of capsid mutations in PLWH-2 failing a LEN-containing regimen. These mutations occurred at positions close to those previously reported in HIV-1. Additional data on the impact of these mutations on the phenotypic susceptibility to LEN are needed. This preliminary study underscores the low genetic barrier to resistance of LEN, especially when the GSS of the optimized background regimen is low, and the need to use it along with therapeutic drug monitoring and therapeutic education.

<table>
<thead>
<tr>
<th>ID</th>
<th>Month post-LEN initiation</th>
<th>Plasma viral load (copies/mL)</th>
<th>GSS of the Optimized Background Regimen</th>
<th>Ameno acid in the HIV-2 capsid</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>Q</td>
</tr>
<tr>
<td>#2</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>Q</td>
</tr>
<tr>
<td>#3</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>Q</td>
</tr>
<tr>
<td>#4</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>Q</td>
</tr>
<tr>
<td>#5</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>Q</td>
</tr>
<tr>
<td>#6</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>Q</td>
</tr>
<tr>
<td>#7</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>Q</td>
</tr>
<tr>
<td>#8</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>Q</td>
</tr>
</tbody>
</table>

Table. First detection of capsid mutations in people living with HIV-2 treated with lenacapavir.

1. An A76V mutation was selected at later time points. 2. These individuals had undetectable viral loads after initiating a LEN-containing regimen, before experiencing virological failure.

683 Role of Dolutegravir in the Emergence of the S147G Integrate Resistance Mutation
Marc Wirden, Basma Abd1, Sidonie Lambert-Nicol1, Marie-Laure Chaix1, Anne de Monte1, Brigitte Montes1, Coralie Pallier1, Pantzika Bellecave1, Magali Bouvier-Alias1, Stephanie Raymond1, Sabine Yerly1, Charlotte Charpentier1, Vincent Calvez1, Anne-Genevieve Marcelli1, for the French ANRS MIE Resistance Study Group

Background: Dolutegravir (DTG) is an integrase strand transfer inhibitor (INSTI) with high efficacy and high barrier to resistance. However some resistance mutations (RAMs) can reduce the HIV susceptibility to this drug. While S147G RAM confers high resistance to elvitegravir (EVG) in the Stanford and ANRS resistance algorithms, only STANFORD included it in the set of DTG resistance mutations.

Results: Eighty-eight strains harboring the S147G for the first time were included. The subtypes were B, CRF02, or other in 49 (55.7%), 19 (21.6%) and 19 (22.7%) cases, respectively. The median viral load and CD4 cell count were 5860 copies/mL (IQR 1011-24525) and 412 cells/ mm³ (228-560), respectively.

Abbreviations: GSS - Genotypic Susceptibility Score; LEN - Lenacapavir; M - Month post-initiation.
The INSTI included in the ongoing treatment was DTG (n=42, 48%), EVG (n=32, 36%), raltegravir (n=9, 10%), bictegravir (n=2) and cabotegravir (n=1). Two patients were not receiving INSTI: one drug-na"e patient infected with a multi-resistant virus, and another had received previous INSTI regimens. The median number of other INSTI-RM associated with the S147G was 2 (1.75-3.00) for all 88 patients, 3 (2.0-3.0) for those on DTG, and 2 (1-2) on EVG. Previously, among the 42 patients with emergence of S147G during DTG regimen, 9 were INSTI naive, 6 had received another INSTI without failure, 25 had failed on INSTI but without S147G for 14/25 and without resistance data for 11/25. The INSTI-RM most frequently associated with S147G under DTG were T94A (62% of cases), N155H (59%), E138K (50%), L74I/V (38%), and Q148R (33%). Nine of these 42 strains were considered resistant to DTG BD but fully susceptible to DTG BID according to ANRS algorithm, while they harbored 4 to 6 INSTI-RMs (S147G + L74I/M, E92Q, T97A, T138K, Y143C, N155H, E157Q, or S230R). These 9 patterns were associated with intermediate resistance to DTG according to Stanford algorithm.

Conclusion: In this study, the S147G mutation is mainly identified during DTG regimen failures. In such a context it can be associated with up to 6 other INSTI-RM, including T97A and/or N155H in the majority of cases. Thus the S147G mutation needs to be added to the DTG resistance profile in the ANRS algorithm.

684 HIV-1 Resistance Mutations to Integrase Inhibitors Impair Both Integration and Reverse Transcription

Background: Reverse transcription and integration are key steps of the Human Immunodeficiency Virus type 1 (HIV-1) replication, performed respectively by the viral enzyme’s reverse transcriptase (RT) and integrase (IN). Interactions between these two enzymes are critical. IN improves both reverse transcription early steps and processivity, while the RT enhances the integrase strand transfer activity. The use of integrase strand transfer inhibitors (INSTIs) has led to emergence of resistant viral variants, occurring mostly in the integrase gene. Most of them exhibit an impaired integration compared to wild-type (WT) viruses. However, their impacts on reverse transcription efficiency remain unclear. Our objective was to determine the impact of three of the main INSTI-associated resistance mutation profiles, R263K, N155H and G140S/Q148H on reverse transcription and kinetics of integration.

Methods: We performed in-vitro infections with wild-type and INSTI resistant viruses: R263K, N155H and G140S/Q148H, produced by site-directed mutagenesis. Early and late reverse transcription products were measured by quantitative digital droplet PCR. Kinetics of integration were analyzed using quantitative PCR on integrated forms of HIV DNA at 24- and 72-hours post infection.

Results: R263K mutation had a major effect on reverse transcription (Figure 1A and B) and a weaker impact on integration. N155H mutation strongly affected reverse transcription (Figure 1A and B) and integration. The G140S/ Q148H double mutant profile was associated with a weak impact on reverse transcription (Figure 1A and B) and a more important impact on integration compared to WT.

Conclusion: INSTI resistance mutations alter integration efficiency but also the reverse transcription step. This phenomenon is in accordance with the close interaction between these two enzymes during HIV-1 replication. These observations might contribute to explain the loss of fitness observed in INSTI resistant mutants during virological failure.

685 High-Level Resistance to Integrase Inhibitors Conferred by Mutations Outside Integrase
Yuta Hikichi, Sherimay D. Ablan, Erin Clark, Eric O. Freed National Cancer Institute, Frederick, MD, USA

Background: Second-generation integrase (IN) strand transfer inhibitors (INSTIs) are highly potent antiretroviral compounds that exhibit a high genetic barrier to resistance. Recent clinical studies concluded that some INSTI-treated individuals experience virological failure in the absence of resistance mutations in IN. The aim of the study is to elucidate INSTI resistance mechanisms and pathways.

Methods: One-year passing of HIV-1 was conducted using the SupT1 T-cell line and primary PBMCs with an escalating concentration of the INSTI dolutegravir (DTG). We evaluated the impact of the selected mutations on replication kinetics and viral infection through cell-free virion and cell-cell contact and performed an array of biochemical and structural analyses on the selected mutants.

Results: HIV-1 became resistant to DTG by sequentially acquiring mutations in Env, Gag-nucleocapsid (NC), and, occasionally, IN. By cloning env from the DTG-treated viruses selected in the SupT1 T-cell line or PBMC, we obtained heavily mutated Env clones, 7XEnv and WD-3, respectively. Both Env mutants exhibit faster-than-WD-3 replication in spreading infection. 7XEnv exhibits resistance to multiple classes of antiretrovirals, with the fold resistance being ~2-logs higher for INSTIs than for other classes of drugs. WD-3 confers 5-fold resistance to DTG in PBMC. Viral transmission of 7XEnv through cell-cell contact is more efficient than that of WT. In contrast, WD-3 exhibits more efficient cell-free infection than WT. These results suggest that the selected Env mutations confer resistance to INSTIs by increasing infection capacity through cell-cell transmission or cell-free viral infection. Viral infection over a range of multiplicities of infection (MOI) revealed that INSTIs are more readily overwhelmed by high MOI than other classes of drugs, leading to high-level resistance to INSTIs. The NC mutations selected with DTG conferred modest (3-5-fold) resistance to INSTIs. Significantly, the NC mutations do not affect cell-free infectivity but accelerate the kinetics of early post-entry events, suggesting that they may limit the window of opportunity for INSTIs to bind intasomes and block integration.

Conclusion: These findings demonstrate that multiple regions in the HIV-1 genome – Env, NC, and IN – collectively contribute to INSTI resistance. The results provide clues to understanding high-level resistance to INSTIs and support the need for genotypic analysis outside of IN in individuals on INSTI-containing regimens.

686 Virological Failure With Cabotegravir-Rilpivirine Injections: A Single-Site Experience
Shivanjali Shankaran, Laura Hernandez-Guarin, Neel Inobalia, Beverly Sha, Mariam Aziz Rush University Medical Center, Chicago, IL, USA

Background: Cabotegravir-rilpivirine (CAB-RPV), the first intramuscular injectable antiretroviral therapy (ART) for people with HIV, is associated with high compliance rates and patient satisfaction. Multiple studies demonstrate low rates of virologic failure (VF) and development of resistance, confirmed by real world analyses. Here we describe our experience with a higher rate of VF at an HIV clinic in Chicago, IL.

Methods: We assessed baseline viral loads (VL) at time of switch in ART, clinic location for the injections and response after transitioning to CAB-RPV. VF was defined as two consecutive VL >200 copies/ml. Resistance mutations in patients with VF were recorded.

Results: 75 undetectable patients (UD, VL <40 copies/ml) were switched to CAB-RPV. 10 received their injections at an independent infusion center (IC) with trained injectors. 65 received injections at our clinic. Two of ten patients at IC and 1 of 65 patients at our clinic developed virologic failure (4%). One patient (pt1) had non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance (K103N), the second (pt2) had an M184V while a genotype failed for the third patient (pt3) at the time of diagnosis. Patients had lived with HIV for 23, 18 and 1 year, respectively, before switch and were lifelong nonsmokers. Pt1 was UD for 2 months and the other two were UD for >6 months prior to switch. All were on an integrate inhibitor (INI) based regimen. Body mass index (BMI) were 27, 35 and 28 respectively. The patients were UD for 6, 10 and 18 months respectively on CAB-RPV before VF. Genotypes at the time of VF revealed INI mutations in pt1: L74I, T97A, S147G/S, N155H, INI and NNRTI mutations in pt2: L74I/A,
Env Conformational Flexibility Modulates HIV-1 Sensitivity to VRC01-Mediated Prevention

Durgadevi Parthasarathy1, Karunakar R. Pothula2, Ruth Parsons2, Xiao Huang2, Salam Sammour2, Katarzyna Janowska2, Miranda Harris1, Joseph Sodroski3, Durgadevi VRC01-Mediated Prevention

1University of Minnesota, Minneapolis, MN, USA, 2Duke Human Vaccine Institute, Durham, NC, USA, 3Dana–Farber Cancer Institute, Boston, MA, USA

Background: HIV-1 envelope glycoproteins (Envs) mediate viral entry and are the sole target of neutralizing antibodies. Envs of most primary HIV-1 strains exist in a closed conformation and occasionally sample more open Env states. Thus, current knowledge guides immunogen design to mimic the closed Env conformation as the preferred target for eliciting broadly neutralizing antibodies (bnAbs) to block HIV-1 entry.

Methods: We evaluated the conformational state of transmitted/founder (T/F) HIV-1 Envs on infectious virions by measuring HIV-1 sensitivity to: 1) antibodies that target internal epitopes, 2) bnAbs, 3) soluble CD4 (sCD4), and 4) exposure to cold. Recognition of soluble HIV-1 Envs (gp120) and surface-expressed Envs by antibodies was measured by ELISA and flow-cytometry, respectively. We solved the cryo-EM structure of an unliganded T/F Env (1059-SOSIP) at 3.6 Å resolution using a large data set and analyzed sub-class structures to estimate the heterogeneity of Env conformations.

Results: We identified T/F HIV-1 Envs that are incompletely closed and sensitive to antibodies that target internal epitopes, to sCD4, and to cold exposure. A cryo-electron microscopy structure of unliganded, incompletely closed T/F Envs (1059-SOSIP), which is resistant to VRC01, at 3.6 Å resolution exhibits an asymmetric configuration of Env protomers with increased sampling of states that are occluded in tightly closed Envs. Recognition of soluble HIV-1 Envs (gp120) and surface-expressed Envs by antibodies was measured by ELISA and flow-cytometry, respectively. We solved the cryo-EM structure of an unliganded T/F Env (1059-SOSIP) at 3.6 Å resolution using a large data set and analyzed sub-class structures to estimate the heterogeneity of Env conformations.

Conclusion: Our findings refine current knowledge of Env conformational states and provide guidance for developing new strategies for bnAb immunotherapy and Env-based immunogen design.

HiResist: A Database of HIV-1 Resistance to Broadly Neutralizing Antibodies

Milind Misra1, Jeffy Jeffy1, Charis Liao1, Stephanie Pickthorn1, Kshitij Wagh1, Alon Herschhorn

1University of Minnesota, Minneapolis, MN, USA

Background: Changing the course of the human immunodeficiency virus type I (HIV-1) pandemic is a high public health priority with approximately 39 million people currently living with HIV-1 (PLWH) and about 1.5 million new infections annually worldwide. Broadly neutralizing antibodies (bnAbs) target vulnerable sites on HIV-1 envelope glycoproteins (Env), which mediate viral entry, and block the infection of diverse HIV-1 strains. But different mechanisms of HIV-1 resistance bnAbs prevent robust application of bnAbs therapeutics and preventative intervention. Here we have developed a specialized database, HiResist (HIV-1 Resistance to bnAbs), to analyze patterns of HIV-1 resistance to different bnAbs. HiResist is freely available online (at hiresist.umn.edu) and is a comprehensive online resource with analysis and visualization tools designed to support the HIV-1 research community, scientists, and the public.

Methods: HiResist is a Flask web application with Gunicorn production server and Apache reverse proxy and is written in Python (50%) and HTML/CSS/JavaScript (50%). Version control is achieved by using private GitHub repositories. The HiResist web server resides on a Linux virtual machine having eight 2.20 GHz Xeon E5-260 processors and 16 GB RAM. Data retrieved from the CATNAP database (www.hiv.lanl.gov) are stored in a periodically updated SQLite database.

Results: HiResist is a bioinformatics tool that allows identification of patterns of resistance and mechanisms of HIV-1 escape; comparison of resistant and sensitive HIV-1 strains for each bnAb; identification of resistance and sensitivity signatures associated with specific bnAbs or groups of bnAbs; and visualization of antibody pairs on cross-sensitivity plots. Additionally, several graphical interfaces are available including heatmaps to cluster selected sets of HIV-1 strains and antibodies, and alignment tools with output displayed in a standard HiResist format, and visualization of resistance/sensitivity signatures of HIV-1 Envs.

Conclusion: In addition to the tools mentioned previously, we continue to develop HiResist and plan to add several new interfaces including tools for comparison of bnAb resistance/sensitivity in HIV-1 strains from different populations of PLWH (e.g., drug users or elite controllers) and tools for assessment of emerging HIV-1 strains. HiResist is being developed to encourage engagement and exploration without the need for programming expertise of these highly relevant data by the broader scientific community.
Development of Individualized Antibody Treatment Regimens for Patients With Multidrug-Resistant HIV

M. A. Rai, Jana Blazkova, Jesse S. Justement, Victoria Shi, Brooke D. Kennedy, Maegan R. Manning, Mary McLaughlin, Michael C. Sneller, Alice K. Pau, Susan Moir, Tae-Wook Chun

National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

Background: People living with HIV (PLWH) who harbor multidrug-resistant (MDR) viruses have limited therapeutic options and present extensive challenges in clinical management. We examined the capacity of HIV-specific broadly neutralizing antibodies (bNAbs) and anti-CD4 antibodies to suppress infectious viral isolates derived from PLWH with MDR viruses.

Methods: We conducted immunologic and virologic analyses on 11 PLWH with MDR viruses. We measured the intact HIV proviral DNA burden and examined levels of immune activation and exhaustion markers by flow cytometry. For comparison, we included a control group of 27 ART-naïve viremic PLWH. We determined the sensitivity of infectious viral isolates obtained from the participants against 8 bNAbs (3BNC117, 10-1074, VRC01, VRC07, N6, 10E8, PGDM1400, and PGT121) and 2 anti-CD4 antibodies (ibalizumab and anti-domain 1 CD4 antibody UB-421) using a TZM-bl-based neutralization/suppression assay.

Results: There was no significant difference in plasma viremia between the study participants with MDR HIV and the control group (P=0.2929). However, the CD4+ T cell counts of the MDR HIV group were significantly lower than those of the control group (P<0.0001). The level of intact HIV proviral DNA was comparable between the two groups (P=0.5895). Levels of activation and exhaustion markers PD-1 (P=0.0019), TIGIT (P=0.0222), 2B4 (P=0.0015), CD160 (P=0.0015), and CD38+/HLA-DR+ (P=0.0138) were significantly lower in the CD8+ T cells of the MDR HIV group. The infectious viral isolates from each study participant with MDR HIV were resistant (IC50 >10µg/ml or % suppression <80.7%) to at least 2 bNAbs (average 4 bNAbs per participant); however, they were sensitive to at least one of the CD4-binding and non-CD4-binding site antibodies. The majority of study participants had ibalizumab-sensitive viruses although the isolates from some participants showed reduced sensitivity to the antibody. Notably, none of the 9 infectious viral isolates obtained from the study participants were resistant to UB-421.

Conclusion: The data from our study has direct clinical implications. Our data suggest that the therapeutic options for heavily treatment-experienced PLWH could be potentially expanded to include HIV-specific bNAbs and UB-421, an avenue that has not been explored until now.

Teropivamib and Zinliviramib Sensitivity in People Living With MDR HIV-1: PRESTIGIO Registry Data

Vincenzo Spagnuolo, Laura Galli, Ayappa Parvanga, Keith J. Dunn, Filippo Lagi, Roberta Gagliardi, L. Sarmati, Annamaria Cattelan, Andrea Giacomelli, Maria Mercedes Santoro, Maurizio Zazzal, Christian Callebaut, Antonella Castagna, Laurie VanderVeen

San Raffaele Scientific Institute, Milan, Italy; Celad Sciences, Inc, Foster City, CA, USA; Azienda Ospedaliero Universitaria Careggi, Firenze, Italy; Lazzaro Spallanzani National Institute for Infectious Diseases, Rome, Italy; University of Rome for Vignola, Rome, Italy; University of Padova, Padova, Italy; Luigi Sacco University Hospital, Milan, Italy; University of Siena, Siena, Italy; San Raffaele Vita-Salute University, Milan, Italy

Background: Broadly neutralizing antibodies (bNAbs) are being investigated as long-acting antiviral therapies, but sensitivity to bNAbs in persons with multidrug-resistant HIV is unknown. Here, we characterized sensitivity to teropivamib (GS-5423, 3BNC117-LS, TAB) and zinliviramib (GS-2870, 10-1074-LS; ZAB) in people living with 4-class drug-resistant HIV (4DR-PWH).

Methods: Multicenter, observational study using plasma or peripheral blood mononuclear cells collected from 50 4DR-PWH (25 with HIV-1 RNA > 1000 copies/ml matched by age, sex, nadir CD4+ and years on ART to 25 virologically suppressed [HIV-1 RNA < 50 copies/mL] enrolled in the PRESTIGIO Registry (NCT04098315) with a documented 4DR (NRTI, NNRTI, PI and INSTI). Phenotypic sensitivity to bNAbs was determined using the PhenoSense Monoclonal Antibody assay (Monogram), with susceptibility defined as IC50 ≤2 µg/mL. Descriptive statistics are used to present results. Spearman's rank test used for associations between phenotypic susceptibility and clinical variables.

Results: Characteristics of included individuals with analyzed samples were indicative of extensive treatment history (Table1). Of 46/50 (92%) participants with PhenoSense mAb assay results, 35 (76%) were phenotypically sensitive to TAB, 23 (50%) to ZAB, and 19 (41%) to both bNAbs; 7 (15%) had phenotypic resistance to both bNAbs. Of 22 viremic participants, 19 (86%) were phenotypically sensitive to TAB, 10 (45%) to ZAB, 9 (41%) to both bNAbs, and 2 (9%) to neither. Of 24 participants with virologic suppression, 67% were phenotypically sensitive to TAB, 54% to ZAB, 42% to both bNAbs, and 5 (21%) to neither. The proportion of participants with sensitivity to both bNAbs was similar (P=0.99) in viremic participants (9/22 [41%]) compared to those with virologic suppression (10/24 [42%]). Nonsignificant correlations between phenotypic sensitivity to bNAbs and age, years of ART, CD4+ cell count, HIV-RNA, type of ART regimen at the sample collection, viral tropism and HIV subtype. There were marginal correlations between phenotypic sensitivity to TAB and years since HIV diagnosis (Spearman r=0.287, p=0.053) and phenotypic sensitivity to ZAB and CD8+ cell count (Spearman r=-0.317, p=0.049).

Conclusion: A significant number of the analyzed 4DR-PWH were found to have virus susceptible to TAB and ZAB. These data provide proof-of-concept that selected multidrug-resistant PWH may be candidates for future trials investigating bNAbs-containing regimens to achieve or maintain virologic suppression.
692 Studying Dual Role of Glycosylation in Resistance to Broadly Neutralizing Antibodies In Vitro

Teresa Murphy, Rebecca Lynch, Gabe Galeotus
George Washington University, Washington, DC, USA

**Background:** Broadly neutralizing antibodies (bNAbs) provide a useful tool for HIV cure strategies because of their ability to target conserved regions on the envelope (Env) protein in the context of both virions and infected cells. One of the most well studied bNAbs is the CD4 binding site (CD4s) antibody, VRC01 and related antibodies. Multiple clinical trials inducing VRC01 into people living with HIV (PWH) demonstrated transient viral suppression. The major obstacle to more effective treatment with bNAbs continues to be viral escape. A deeper understanding of escape pathways from VRC01-class antibodies in genetically diverse samples is needed.

**Methods:** We developed an in vitro viral escape assay to test bNAb and Env combinations. Ex vivo CD4+ T cells were infected with infectious molecular clone 246.F3-NL4.3 (AC) in the presence of varying concentrations of VRC01. Cultures were maintained with suboptimal concentrations of NAbs to induce escape. Replication kinetics were monitored by p24 every 3 days. Every 14 days, target cells were replenished and cultures tested for genotypic and phenotypic measures of bNAbs resistance. This was accomplished by single genome sequencing envs and by TZM-bl neutralization assay. Individual mutations were then tested for their contribution to resistance to CD4bs bNAbs by pseudovirus neutralization assay using mutated env plasmids.

**Results:** Using our viral escape assay, we observed both previously published and novel escape mutations. Complete resistance to VRC01 was detected in 246.F3 by day 45. A mutation at position N276 that eliminated the glycan conferred complete resistance to VRC01, despite canonically increasing sensitivity. To study the neutralization profile of this mutation in various subtypes, it was inserted into 12-virus global panel of envs and tested for sensitivity compared to wildtype against a panel of CD4bs bNAbs. This mutation was shown to both increase resistance or sensitivity depending on the envelope and bNAb in question, emphasizing a dual role of this glycan in VRC01 class neutralization.

**Conclusion:** Our data demonstrate that our viral escape assay can highlight novel pathways, such as the loss of glycan 276 conferring complete resistance to VRC01 in 246.F3.env. The role of this glycan in escape was demonstrated to be dependent on both the context of the env as well as the bNAb. This finding emphasizes the importance of studying viral escape with a genetically diverse library to develop a deeper understanding of various pathways.

**Figure 1. N276D Mutation Sensitivity to VRC01**

**Table 1. Characteristics of individuals with an analyzable sample at the time of sample collection (n = 40).**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>52.3(21.6 - 85.1)</td>
</tr>
<tr>
<td>Males</td>
<td>28 (60.0%)</td>
</tr>
<tr>
<td>Time since HIV diagnosis, years</td>
<td>25.1(22.3 - 58.0)</td>
</tr>
<tr>
<td>Time since ART start, years</td>
<td>23.1(19.0 - 29.0)</td>
</tr>
</tbody>
</table>

**Note:** Values are presented as the mean (range).
Longitudinal Analysis of Preexisting Resistance-Associated Mutations

Prior to B/F/TAF Switch
Michelle L. D’Antoni, Kristen Andreotta, Silvia Chang, Jason Hindman, Laurie VanderVeen, Christian Callebaut
Gilead Sciences, Inc, Foster City, CA, USA

Background: Preexisting resistance can affect antiretroviral (ARV) efficacy. Circulating HIV-1 variants with drug resistance-associated mutations (RAMs) can be archived in viral reservoirs, where they can persist and re-emerge. Given the dynamic properties of the latent reservoir, detection of these RAMs over time has not been well defined.

Methods: Participants from bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) switch studies (4445, 4030, 4580, virologic suppression ≥6 months; no prior virologic failure) with HIV-1 RNA or proviral HIV-1 DNA genotyping (population reporting) from ≥2 pre-switch timepoints were included. For 2 timepoints only, RAMs in protease, reverse transcriptase, and other substitutions (accessory, polymorphisms) were categorized as detected 100% (2 out of 2 tests) or 50% (1 out of 2 tests; subcategorized as detection lost or gained) of the time. For >2 timepoints, RAMs were categorized as lost, gained, persistent, or fluctuating. Time between first and last tests was reported in median years (y). Nonparametric statistics were used.

Results: In all, 235 participants had evaluable data (longitudinal tracking of ≥1 RAM or other substitution). For 206 participants with ≥2 timepoints, 103 of 256 RAMs reported (40.2%) had 100% detection, and 153 (59.8%) had ≥50% detection, with 95 (62.1%) gained and 58 (37.9%) lost (Figure). 100% detection was lower for RAMs versus other substitutions (n=3329/4339 ≥1 RAM or other substitution). For 206 participants with ≥2 timepoints (median 3 reports, 9.3 y [IQR 5.7-13.9] between first and last tests), 66 RAMs were categorized as fluctuating (48.5%), gained (25.8%), persistent (15.2%), or, least common, lost (10.6%). K103N (n=10) and M184VI (n=16) were predominantly fluctuating (60.0% and 43.8%, respectively) and only lost in 10.0% and 6.3% of cases, respectively.

Conclusion: Some RAMs were consistently reported, but the majority were newly detected or fluctuated and did not disappear significantly over time, which most likely reflects both the ongoing decay and proliferation of the latent reservoir and ARV pressure. RAMs were not always detected, and this lack of longitudinal stability enforces the need to consider an individual’s treatment history and all past genotyping test results (and the detection sensitivities of reports) for treatment management.

A Phase II Trial of 4 Weeks of Glecaprevir/Pibrentasvir for Early Hepatitis C Virus: ACTG A5380

Arthur Kim, Minhee Kang, Triin Umbloja, Estevao P. Nunes, Kristen Marks, Channelle Wimbishi, Daniel S. Fierer, Annie Luetkemeyer, Dimas Kleimann, Sunil Suhais Solomon, Leonard Sowah, Beverly L. Atston-Smith, David L. Wyles, Susanna Naggiat, for the A5380 Study Team

Background: Shorter treatment courses have been effective in early hepatitis C (HCV) but are still longer than optimal. Shortening from 8-12 weeks to a single month simplifies treatment and may further facilitate the national plan of HCV elimination.

Methods: A5380 was a prospective, phase II, single-arm multicenter trial evaluating the efficacy and safety of once daily oral glecaprevir/pibrentasvir (G/P) 300 mg/120 mg for 4 weeks in adults with early HCV. Early HCV was defined as new ALT elevation (≥5x ULN or >250 U/L if normal ALT in prior year, or ≥50% decrease or <150 U/L if normal ALT in prior year) or detectable HCV RNA with prior negative antibody (1st infection) or HCV RNA (re-infection) within 24 weeks prior to study entry. The primary endpoint was sustained virologic response, SVR12, defined as HCV RNA ≤LLOQ at 12 weeks after treatment cessation. A5380 was powered to conclude that the SVR12 proportion is >80% using the 90% Wilson confidence interval (CI). Participants not achieving SVR12 were offered re-treatment.

Results: Forty-five participants (98% male, 51% White, 27% Black, 31% Hispanic/Latino, median age 36 years (range 22-65) were enrolled from the U.S. and Brazil between Nov 2019 and Jan 2023; 27% reported a history of injection drug use, 84% were 1st HCV infections. 51% were people with HIV (PWH), with CD4 / HIV RNA reported in Table 1. Median time from diagnosis to entry was 31 days (IQR: 15-49). Median baseline HCV RNA was 5.3 log IU/mL (IQR: 3.3-6.0), 71% genotype 1; median ALT was 146 U/L (range: 22-3866). SVR12 was achieved in 38 of 45 (84%) participants (CI: 74%-91%) and 86% (CI: 76%-93%) excluding 1 participant lost to follow-up (LFU). SVR12 was 83% (CI: 66%-92%) in PWH and 86% (CI: 70%-94%) in those without HIV. There were no treatment-related serious adverse events. For the 6 participants with recurrent viremia at or before SVR12, median baseline HCV RNA was 6.3 log IU/mL (IQR: 3.8-71) and self-reported pill counts suggested good adherence. Re-treatment with salvage regimens resulted in SVR12 for 4 of 4 participants with treatment follow-up.

Conclusion: Treatment of early HCV with 4 weeks of G/P resulted in clinically acceptable cure rates in people with or without HIV. Although SVR12 >80% could not be concluded from the CI, a planned analysis excluding LFU supports that the SVR12 proportion is >75%. As simplified treatment approaches are critical for HCV elimination, a 4-week G/P regimen should be tested in implementation programs aiming to cure early HCV.
697 A Precision Randomized Trial of Hepatitis C Treatment Adherence Support Among 3000 PWID in India
The Johns Hopkins University, Baltimore, MD, USA, *YR Gaitonde Center for AIDS Research and Education, Chennai, India

Background: HCV elimination is unlikely without curing people who inject drugs (PWID). Many PWID will need additional support to achieve HCV cure, but resources are limited, particularly in low- and middle-income countries. A precision approach could improve the efficiency of intervention delivery. The STOP-C trial evaluated whether HCV treatment outcomes could be optimized by tailoring adherence support to PWID need across community-based centers in 7 cities across India.

Methods: We implemented a precision randomized trial where arm assignment probabilities varied by participants’ estimated probability for HCV treatment failure. A prediction model generated a prognostic score that classified persons as low or high-risk for failure. Those at high-risk were randomized 3:2:1 to patient navigation + flexible directly observed therapy with ≥ 1 weekly dose observed (PN+DOT), PN only, or basic support. Those at low-risk were randomized 1:2:3 to PN+DOT, PN only or basic support. All had a history of drug injection and were treatment naive; those with compensated decompensated cirrhosis were excluded. All received sofosbuvir/velpatasvir twice daily for 12 weeks. The primary outcome, sustained virologic response (SVR), HCV RNA < LLOQ 12 weeks after treatment completion was compared by Poisson regression adjusted for site (intent to treat, missing = failure).

Results: 3000 participants were recruited from Jan 2021-Dec 2022 (2048 low-risk, 952 high-risk). Compared with participants in the low-risk stratum, those in the high-risk stratum were more likely to be younger (median 27 vs. 31), experience homelessness (26% vs 6%) and report active drug injection (89% vs. 42%). 2798 (93%) completed SVR assessment. 49% and 63% achieved SVR in high and low-risk strata, respectively. In the high-risk stratum, SVR in PN+DOT was similar to PN only and basic support. In the low-risk stratum, SVR in PN+DOT was associated with a statistically significant 9% increase in SVR vs. basic support (adjusted relative risk [aRR] 1.09; p=0.04) but did not differ in PN only vs. basic support. Within strata, significantly stronger associations with better prognostic score (aRR per 10% decrease: 1.04 low-risk, 1.10 high-risk; p<0.01 for both).

Conclusion: Greater adherence support or long-acting treatments may be required to improve cure rates among PWID at highest risk for failure. However, programs could use prognostic scores to target interventions more efficiently and proactively address barriers to treatment adherence.

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698 Prevalence and Risk Factors for Hepatitis C Viremia in People With HIV in the US During the DAA Era
Joanne Carson, Marianne Martineille, Samira Hosseini-Hooshybar, Phillip Read, David A. Baker, Jeffrey Post, Robert Finlayson, Mark Bloch, Joseph Doyle, David Shaw, Margaret Hellard, Eceatare Filipe, Gregory Dore, Gail Matthews
*University of New South Wales, Sydney, Australia, †University of Sydney, Australia, ‡The Albion Centre, Sydney, Australia, ††Taylor Square Private Clinic, Sydney, Australia, †‡Burnett Institute, Melbourne, Australia, †§Royal Adelaide Hospital, Sydney, Australia, †¶St Vincent’s Hospital Sydney, Sydney, Australia

Background: The prevalence of HCV viremia during the DAA era in this US-based national cohort of PWI improved over time and across demographic subgroups but remains elevated. Our findings underscore the importance of prioritizing substance use and mental health treatment in PWH to achieve HCV elimination goals.

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699 Elimination of HCV Among People With HIV in Australia
Joanne Carson, Marianne Martineille, Samira Hosseini-Hooshybar, Phillip Read, David A. Baker, Jeffrey Post, Robert Finlayson, Mark Bloch, Joseph Doyle, David Shaw, Margaret Hellard, Eceatare Filipe, Gregory Dore, Gail Matthews
*University of New South Wales, Sydney, Australia, †University of Sydney, Australia, ‡The Albion Centre, Sydney, Australia

Methods: Using a serial cross-sectional design, we examined all adult PWH in clinical care at 9 sites in the CFAR Network of Integrated Clinical Systems (CNICS) cohort with ≥1 encounter in 2011-13 (pre-interferon free DAA era, number of participants=22445), 2014-17 (early DAA era, N=25161), or 2018-22 (current DAA era, N=25314). We defined HCV viremia as a positive HCV RNA or genotype. Prevalence of HCV viremia was determined for each time period, using the most recent available lab results, and by demographic group (age, gender, race/ethnicity). We administered validated survey instruments to measure current substance use (AUDIT-C; alcohol; ASSIST, other drugs) and depressive symptom severity (PHQ-9). FIB-4 score was calculated using the age and closest lab values on or before a participant’s HCV lab test. We used adjusted relative risk regression (Poisson) to evaluate risk factors for HCV viremia in 2018-22.

Results: Among PWH in care, the overall prevalence of HCV viremia was 8.7% in 2011-13, 10.5% in 2014-17, and 4.8% in 2018-22. Disparities in the prevalence of HCV viremia across groups defined by age, gender, and race/ethnicity were smaller in 2018-22 than earlier time periods (overall range across all 3 demographic groups by time period: 3.0-13.0% [2011-13]; 3.5-14.4% [2014-17]; 3.2-5.6% [2018-22]). In adjusted relative risk regression, being female (RR 1.33, 95% CI 1.13-1.56), FIB-4 (RR 1.67 per unit, 95% CI 1.59-1.76), depression symptom severity (RR 1.12 for 5 units on PHQ-9, 95% CI 1.08-1.30), and current use of methamphetamine (RR 2.76, 95% CI 2.06-3.68) or illicit opioids (RR 2.22, 95% CI 1.58-3.12) (P<0.001 for each factor) were associated with higher likelihood of HCV viremia in 2018-22.

Conclusion: The prevalence of HCV viremia during the DAA era in this US-based national cohort of PWI improved over time and across demographic subgroups but remains elevated. Our findings underscore the importance of prioritizing substance use and mental health treatment in PWH to achieve HCV elimination goals.

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were calculated per 100 person-years (PY). Cox regression was used to assess associated factors.

**Results:** Of 314 participants (median age 49 years, 97% male [89% gay or bisexual]), 13% cirrhosis, 80% history of injecting drug use (IDU), 42% current IDU (past month) data were available for 295 at FU1 and 266 at FU2 (220/266 HCV RNA available). Of those with detectable HCV RNA at ENR (n=224), 210 received HCV treatment, 204 had documented cure, and 189 had post-cure visit. The proportion with detectable HCV RNA declined from 71% (ENR), to 7% (FU1), to 1% (FU2). Fourteen participants had HCV reinfection (13/14 retreated). HCV reinfection rate was 1.5/100 PY (95% CI 0.10, 2.60; median FU 5.6 years); decreasing from 2.7/100 PY (ENR-FU1) to 0.60/100 PY (FU1-FU2). Current IDU (adjusted hazard ratio [AHR] 3.72 95% CI 1.29, 10.72) increased reinfection risk. In the overall study population, 29 died. Death rate was 2.1/100 PY (95% CI 1.46, 3.01; median FU 4.7 years), stable over time. Median age at death was 56 years (range 29-68). Leading causes of death were cancerous comorbidities (24%), cancer (21%), sepsis (14%), and drug overdose or suicide (7%). Older age (AHR 1.06 per year 95% CI 1.01, 1.11) and liver cirrhosis (AHR 2.46 95% CI 1.10, 5.52) increased death risk. Current IDU did not increase death risk, although younger age (<35 years) at injecting initiation did (AHR 3.56; 1.06, 12.00).

**Conclusion:** HCV prevalence among people with HIV in Australia has declined substantially following rapid DAA scale-up, however surveillance, for HCV (re)infection and associated morbidity and mortality, remains important.

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**Community Pop-Up Clinic: Cascade of Care and HCV Treatment of Vancouver’s Inner-City PWID Population**

Brian Conway, Saina Beitali, Shawn Sharma, Rossissa Yang, Shana Yi

**Vancouver Infectious Diseases Center, Vancouver, Canada**

**Background:** Several strategies have been proposed to identify HCV-infected inner-city residents, engage them in care, provide them with antiviral therapy, to the primary care level and integration into existing services. In 2021, we initiated a Community Pop-Up Clinic (CPC) program, with additional analyses of HCV reinfection events and opioid-related mortality.

**Methods:** We have evaluated a novel approach of Community Pop-Up Clinics and its ability to promote access to care, uptake of HCV therapy and its outcome, with additional analyses of HCV reinfection events and opioid-related mortality. We hypothesized that by implementing this CPC program, we will optimize engagement in care of vulnerable inner-city populations, increase successful uptake of HCV therapy and reduce reinfection events and mortality.

**Results:** From January 2021 – August 2023 (32 months), we conducted 112 CPCs and evaluated 1968 individuals. 620 individuals (31.5%) were found to carry HCV antibodies. Of 620 individuals we identified as carrying HCV antibodies, 474 individuals (76.5%) were found to be viremic. HCV engagement has been secured in 387 cases (81.6%). 326 (84.2%) individuals have started treatment and 60 are in the pre-treatment phase, and 1 had died of an overdose in the pre-treatment phase. The median time from CPC attendance to HCV treatment initiation was 6 weeks. Of 326, 302 have completed treatment, 18 are currently on treatment and 1 died of an overdose during treatment. Of 302 subjects who have completed treatment, 286 are confirmed as cured (SVR 12), 16 are awaiting SVR 4, 2 documented virologic relapse and 1 documented to be reinfected, a rate of 0.31/100 person-years. 3 patients withdrew from the treatment. By mITT, the cure rate is 286/288 (99.3%). Overall, in this vulnerable population with 6-7 opioid overdose deaths/day, we only documented 2 overdose deaths over 326 PY of overall follow-up.

**Conclusion:** Taken together, the data we present validates the development of multidisciplinary programs such as ours aimed at treating HCV in vulnerable inner-city populations that must be engaged in care for HCV elimination to become a reality. This report also documents additional societal benefits, e.g., lower overdose death, that could be achieved from such a program.

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**High Completion and Cure Rates With a Decentralized, Integrated HCV Treatment Approach in Vietnam**

Nhan T. Do1, Nhut T. Vo2, Huong T. Phan3, Dung A. Tran2, Thuy T. Pham1, Chieu V. Vu1, Linh An T. Tong4, Hang T. Duong2, Duy T. Nguyen4, Mai T. Pham4, Luu A. Cosimi4, Todd Pollack5,6

1Vietnam Administration for HIV/AIDS Control, Hanoi, Vietnam, 2Beth Israel Deaconess Medical Center, Boston, MA, USA, 3Beth Israel Deaconess Medical Center, Hanoi, Vietnam, 4The Global Fund, Hanoi, Vietnam, 5Brigham and Women’s Hospital, Boston, MA, USA

**Background:** WHO recommends a simplified service delivery approach to the treatment of chronic hepatitis C (HCV) infection including decentralization to the primary care level and integration into existing services. In 2021, with support from the Global Fund, the Vietnam Administration for HIV/AIDS Control within the Ministry of Health scaled up HCV treatment at public HIV and methadone clinics in 36 provinces.

**Methods:** Patients with HCV viremic infection with and without liver fibrosis or cirrhosis were eligible for HCV treatment through the National program. Patients were treated with sofosbuvir/daclatasvir for 12 or 24 weeks following the Vietnam national guidelines. Program data were collected and stored in the Ministry of Health database. We assessed HCV treatment outcomes at national, provincial and district levels. The primary endpoints were treatment completion and sustained virologic response 12 weeks after treatment completion (SVR12).

Multivariate logistic regression analysis was used to find factors associated with SVR12.
High Rate of Hepatitis C Incidence in Vietnamese MSM Living With HIV

Donn J. Goby, Minh T. Nguyen, Lan A. Do, Tam C. Le, Huu T. Tran, Binh Q. Luong, Phuong K. Doan, Khang Q. Do, Hung Yan, An Bas

1Hanoi Medical University, Hanoi, Vietnam; 2Ho Chi Minh City, Vietnam; 3Center for Applied Research on Men and Community Health, Ho Chi Minh City, Vietnam; 4US Centers for Disease Control and Prevention Ho Chi Minh City, Vietnam; 5Cherokee Nation Health Services, Tahlequah, OK, USA, 6US Centers for Disease Control and Prevention Can Tho, Can Tho, Vietnam; 7Vietnam, 8Vietnam, 9Vietnam, 10Vietnam, 11Vietnam, 12Vietnam

Background: Men who have sex with men (MSM) living with HIV have higher rates of hepatitis C virus (HCV) infection than MSM without HIV infection in North America and Europe. Studies have shown similarly high rates of HCV incidence among MSM living with HIV in large Asian cities, including Bangkok, Taipei, Hong Kong, and Tokyo. There are limited data on HCV incidence among MSM in Vietnam.

Methods: MSM and transgender women (TGW) were recruited at two antiretroviral therapy clinics for people living with HIV in Ho Chi Minh City and Can Tho, Vietnam. Participants provided informed consent at the first visit and had a second visit after 12 months. At each visit, participants competed a questionnaire on risk behaviors (sexual behavior and substance use) and had blood drawn for HCV antibody testing. The study was approved by supervising IRB in Vietnam.

Results: Enrollment included 532 participants; 521 (98%) completed two study visits and were included in the incidence analysis. The study population included 76% MSM and 24% TGW with a median age 27 (IQR 24-30). Three-quarters (75%) had high school education or above, with over half (58%) reporting at least some college education. Reported risk behaviors during the follow-up period included condomless anal/vaginal sex (47%), group sex (29%), and methamphetamine use (21%). Injection of methamphetamine appears to be a newly emerging risk in Vietnam; 3 individuals (0.6%) reported this risk at follow-up while none reported it at baseline. HCV prevalence at baseline was 3.5% (0.6%); all of whom were MSM. At 12 months, 7 new HCV infections were detected, for an incidence of 12.0 per 1,000 person years. All incident cases were among MSM participants. Restricting incidence analyses to only MSM (n=397), the incidence rate was 15.2 per 1,000 person years. In the multivariable logistic regression analysis, the only factor significantly associated with new HCV infection was participating in group sex (adjusted odds ratio (aOR) 13.8, 95% confidence interval 12.9-146.5).

Conclusion: HCV incidence is significant among MSM living with HIV in southern Vietnam. HCV infection appears to be related to sexual activity, particularly group sex, which is also often associated with use of recreational drugs such as methamphetamine. Clinics that provide HIV care to MSM clients should provide prevention counseling to at-risk MSM and regular HCV screening for those with high-risk behaviors.
describe the characteristics of contacted cases and the frequency and correlates of treatment.

Methods: Volunteer study case-workers contacted Los Angeles County residents with a positive HCV RNA test result reported to the Department of Public Health between January 2021 and April 2022 to assess awareness of their infection status, verify treatment, and counsel untreated cases. We evaluated bivariate associations of race/ethnicity, age, biological sex, insurance status (private, public (Medicare, Medical), and none), and symptomatic status (symptoms vs no symptoms) with treatment status (treated vs. untreated) using a Pearson's Chi-Square Test. We created a multivariable logistic regression model to assess associations between demographic and clinical characteristics and treatment status.

Results: Among 403 cases contacted, 227 (56%) had public insurance, 254 (63%) were male, 230 (57%) were 45+ years old, and 181 (45%) were Hispanic or Latino. Eighty-five percent were aware of their positive HCV test result, yet 68.1% never received treatment. Untreated cases (N = 295) were predominantly male (65%) and non-White (17%). No statistically significant differences between treatment status existed for race/ethnicity and sex. The multivariable logistic regression model showed public insurance status (vs private odds ratio [OR]: 0.36; 95% CI: 0.32, 0.39), older age group (vs young adults 18-29 years OR: 3.17, 95% CI: 2.13, 8.18), and the existence of symptoms (vs no symptoms OR: 3.70; 95% CI: 2.15, 6.64) were associated with treatment.

Conclusion: HCV case registry data can be used to inform people about their infection, assess treatment status and counsel untreated cases. Those publicly insured, younger, and asymptomatic were less likely to be treated. Local health departments should use case registry data to help accelerate HCV elimination efforts.

706 A Model to Eliminate Viral Hepatitis Infection in Migrants: A Prospective Study in Southern Italy

Antonio Russo1, Manantonietta Pisaturo2, Alessio Loredana1, Stefania De Pasquali3, Margherita Macera1, Vincenzo Messina3, Lorenzo Onorato4, Carmine Michlini3, Maria Stanzione4, Gianfranca Stornaiolo4, Mario Starace1, Caterina Monari5, Caterina Sagnelli1, Nicola Coppola1

1University of Campania Luigi Vanvitelli, Naples, Italy, 2Amendola Sant’Annea e San Sebastiano di Caserta, Caserta, Italy

Background: Migrants born in intermediate and high HBV and HCV-prevalence countries are likely to be at an increased risk for HBV and HCV infection. Data on HBV and HCV prevalence in migrants living in Italy is scanty and there are few screening and linkage-to-care programs for this target.

Methods: A prospective, multicenter, based on the long-term active cooperation between two 3rd level units of Infectious Diseases and four 1st level clinical centers in southern Italy (Naples and Caserta) was designed. The study started in June 2018, was stopped in February 2020, and was resumed in February 2021 until November 2021. All migrants > 18 years old consecutively evaluated for clinical consultation at one of the first-level centers were enrolled. An anonymous serological screening was offered to seek HCV, HBV and HCV. The participants who were positive for a virus hepatitis infection and or for HCV were referred for linkage to care at one of the tertiary units.

Results: In the study period we observed 3,501 migrants; 3,417 (97.6%) agreed to be screened. Of the 3,417 subjects screened 185 (4.7%) were anti-HCV-positive, 334 (10%) were HBsAg positive, 61 (1.7%) HIV Ab positive. Of the 334 HBsAg positive subjects (figure 1), 116 (35%) had HBV DNA over than 2000 UI/ML. Of the 116 subjects with HBV DNA over than 2000 UI/ML, 111 (96%) had chronic hepatitis, 3 (2%) had cirrhosis and 2 (1.7%) had HCC; all subjects with HBV DNA over than 2000 UI/ML were linked to care but 3 (2.6%) lost to follow up. Eight subjects (2%) were HDV Ab positive, but only one were HDV RNA positive, genotype 1 and was linked to care. Of the 185 HCV ab subjects, 53 (29%) were HCV-RNA-positive. Of the 53 HCV-RNA-positive-subjects, 48 (90%) were linked to care, 5 (10%) refused. Of these 48, 16 (33.3%) harboured HCV genotype 1b, 11 (22.9%) genotype 1a, 16 (33.3%) genotype 3, 3 (6.3%) genotype 4 and 2 (4.2%) genotype 2. All the 48 HCV-RNA-positive patients started DAAs-regimen with sofosbuvir/velpatasvir and completed the 12 weeks of treatment. Of these 48 subjects, 47 (97.9%) showed a sustained virologic response (SVR) at 12 and at 24 weeks after treatment and one dropped-out in follow-up after finishing the DAAs treatment.

Conclusion: After an educational phase on the route of transmission and treatment availability, nearly 98% of subjects agreed to be screened and evaluated for hepatitis virus infections, so our model seems useful in the viral hepatitis screening, linkage-to-care and treatment in a difficult to manage population.

707 Hepatitis C Screening in the Emergency Department: The Multi-Center DETECT Hep C Clinical Trial

Jason Haukoos1, Sarah E. Rowan2, Emily Hopkins2, James Galbraith3, Richard E. Rothman4, Yu-Hsiang Hsieh5, Stephanie Gravitz6, Kevin Ramis6, Carolynn Lyle1, Michael S. Lyons1, Douglas White7, Ali Al-Tayyib8, Edward Gardner9, David L. Wyles1, for the DETECT Hep C Screening Trial Investigators

1Denver Health Medical Center, Denver, CO, USA, 2University of Mississippi Medical Center, Jackson, MS, USA, 3The Johns Hopkins University, Baltimore, MD, USA, 4Denver Health and Hospital Authority, Denver, CO, USA, 5The Ohio State University, Columbus, OH, USA, 6Highland Hospital, Oakland, CA, USA

Background: Testing for hepatitis C (HCV) is the first step toward ultimately curing HCV infection and preventing transmission. Emergency departments (EDs) are key clinical settings for screening given that they serve at-risk patients who commonly do not access healthcare elsewhere. Determining the best approach to HCV screening in ED settings is imperative to maximize the benefit of this public health intervention. The goal of this study was to evaluate the effectiveness of HCV screening in EDs with the hypothesis that nontargeted screening is significantly associated with identification of new diagnoses when compared to targeted screening.

Methods: Design & Setting: Prospective multi-center randomized pragmatic trial in EDs in Denver, CO, Baltimore, MD, and Jackson, MS. Population: Patients ≥18 years of age presenting for ED care. Exclusions: critical illness, inability to consent, prior HCV diagnosis. Interventions: Eligible patients underwent concealed randomization as part of routine care to either nontargeted screening, where all were offered HCV testing, or targeted screening, where all were asked risk questions and any affirmative response led to an offer for HCV testing. Screening was fully integrated into ED care using opt-out consent; antibody positive tests were followed by reflex RNA testing. Outcomes included newly diagnosed HCV (RNA-detected) and elements of the HCV care continuum through 12 months of follow-up. Analyses: Intention-to-treat, chi-square, and risk ratios (RRs) with 95% confidence intervals (CIs).

Results: From 11/19/2019 through 8/4/2022, 147,498 eligible patients were randomized with excellent balance of characteristics. Of the 73,651 allocated to targeted screening, 23,339 (31.9%) were identified as high risk, 7,110 (30.4%) accepted testing and 4,634 (65.3%) completed testing, resulting in 114 (2.5%) new diagnoses. Of the 73,847 allocated to nontargeted screening, 16,516 (22.5%) accepted testing and 9,825 (59.5%) completed testing, resulting in 154 (1.6%) new diagnoses. Compared to targeted screening, nontargeted screening was significantly associated with new diagnoses (RR 1.35, 95% CI 1.06-1.72, p = 0.02). Small proportions had treatment appointments, initiated and completed treatment, or attained SVR12 (Figure).

Conclusion: Nontargeted HCV screening was superior to targeted screening for identifying newly diagnosed HCV in the ED. The significant decay from diagnosis to SVR12 suggests innovative models of HCV treatment from the ED are needed.
Navigation is Superior to Clinician Referral for Linkage to HCV Care from the Emergency Department


Denver Health and Hospital Authority, Denver, CO, USA, 1University of Colorado Denver, Denver, CO, USA.

Background: Emergency departments (EDs) serve as important clinical settings for hepatitis C (HCV) screening and care, yet optimal methods of linkage-to-care for HCV-diagnosed individuals remain unknown. The goal of this study was to test the effectiveness of linkage navigation (LN) and clinician referral (CR) among ED patients identified with untreated HCV with a primary hypothesis that LN plus CR is superior to CR alone.

Methods: We performed a prospective two-arm parallel-group comparative effectiveness randomized trial at Denver Health Medical Center among ED patients with untreated HCV. Participants were randomized in a concealed fashion to CR alone or CR plus LN. All participants provided informed consent. Individuals in the CR arm were educated about HCV by their primary ED clinician and given information on how to access HCV care verbally and in discharge instructions. Individuals in the LN arm met with a linkage navigator in the ED or by phone after the visit. The LN reintegrated basic HCV education and helped schedule and facilitate HCV treatment appointments. Pre-specified outcomes, collected in a blinded manner, were initiation of HCV treatment (primary), appointment with an HCV clinician, completion of treatment, and sustained virologic response 12 weeks after treatment (secondary) at 6 months post-enrollment. Analyses were performed using intention-to-treat with differences, precision estimates, and bivariate hypothesis testing.

Results: From November 2019 through January 2023, 280 individuals were randomly assigned with excellent balance in baseline characteristics, including individuals <40 years of age or with recent injection drug use (IDU). Individuals in the LN arm were significantly more likely to link to care (Δ = 18%, p = 0.0004) and initiate treatment (Δ = 11%, p = 0.01). More individuals in the LN arm completed treatment though this was not statistically significant (Δ = 7%, p = 0.09) (Table). Among participants <40 years or with recent IDU, treatment completed treatment though this was not statistically significant (Δ = 7%, p = 0.09). Among participants <40 years or with recent IDU, treatment initiation did not differ between arms (p = 0.75).

Conclusion: Among ED patients with untreated HCV, early follow-up suggests that LN in addition to CR is superior to CR-alone for initiating HCV treatment, but additional follow-up is needed to better understand the effect of a linkage navigator on the downstream elements of the HCV continuum, including treatment completion and cure, and cost effectiveness of employing a navigator. Even with a navigator, linkage rates were low suggesting new approaches to HCV treatment initiation are needed.

Table 6-month outcomes for patients with untreated HCV enrolled in the DETECT Hep C Linkage-to-Care Trial

<table>
<thead>
<tr>
<th>Linkage Referral</th>
<th>Non-targeted</th>
<th>Targeted</th>
<th>Non-targeted</th>
<th>Targeted</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = 403 (95% CI)</td>
<td>36 (8.8-8.8)</td>
<td>39 (9.5-9.5)</td>
<td>35 (8.3-8.3)</td>
<td>39 (9.5-9.5)</td>
</tr>
<tr>
<td>Δ (%)</td>
<td>11 (95% CI)</td>
<td>11 (95% CI)</td>
<td>11 (95% CI)</td>
<td>11 (95% CI)</td>
</tr>
<tr>
<td>p</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
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</table>

Linkage-to-care for Hep C

Linked to care for Hep C 46 (32.4%) 20 (14.5%) +17.9 (4.2-27.4) 0.0004

Initiated Hep C treatment 31 (23.1%) 15 (10.9%) +11.0 (2.4-19.5) 0.01

Completed Hep C treatment 24 (16.9%) 14 (10.1%) +6.5 (1.2-14.7) 0.19

Documentation S/P/H 21 (22.1%) 33 (28.6%) 0.16

Abbreviations: CI, confidence interval; Hep C, hepatitis C; S/P/H, sustained virologic response 12 weeks *S/P/H defined as an undetectable HCV RNA 12 weeks after completing treatment.

709 Peripartum Linkage to Care in Hepatitis C: Infant Testing and Maternal Treatment

John Cafardi, Hong Lin, Lana Lange, Lacey Kelley, Kelly Leman, Elisabeth Odegard, Heidi L. Meeds, Jason T. Blackard, Judith Feinberg

The Christ Hospital, Cincinnati, OH, USA, 1University of Cincinnati, Cincinnati, OH, USA, 1West Virginia Clinical and Translational Science Institute, Morgantown, WV, USA.

Background: There is an increase in hepatitis C virus (HCV) infection due to injection drug use and poor access to care. Ohio had an 89% increase in HCV in women of childbearing age between 2010 and 2015 with children born to HCV-infected women increased 68%. HCV testing of infants is recommended at 18 months of age but testing and follow-up are poor. Between 2011-2013 only 16% of eligible infants in Philadelphia received appropriate testing. Maternal linkage to care and treatment are also inadequate, with rates commonly less than 20% improvements in linkage to care as well as testing and treatment are needed.

Methods: Fifty-four pregnant women with chronic HCV infection were recruited from outpatient clinics in Ohio and West Virginia. Eligible participants were 18 or older with singleton pregnancy up to 36 weeks. Hepatitis B coinfection, active drug use and severe medical comorbidities were exclusionary. After written informed consent, participants were educated about HCV and were seen up to seven times (enrollment, 36 weeks gestation, and 12, 24, 36 and 48 weeks postpartum). All participants were offered once daily sofosbuvir-velpatasvir for 84 days at 24 weeks postpartum. Cessation of breastfeeding and negative pregnancy test were confirmed prior to treatment. Three maternal blood samples were collected (36 weeks as well as at 28- and 48-weeks postpartum) while infant blood samples were collected at 12, 24, and 48 weeks postpartum. All maternal and infant testing was performed simultaneously at co-localized appointments.

Results: Data were available for 49 mothers and 30 infants. All had a history of injection drug use; none reported use during the study. Fifty-two were White and two were Black per self-report; one participant was co-infected with HIV. Due to non-adherence, placement in foster care, and COVID19 related shutdowns, 23 of 30 (77%) of infants were tested, yielding 23 evaluable mother-infant pairs. Of these, 5 infants (22%) completed three study visits, 7 (30%) completed two visits, and 11 (48%) completed one visit. 28 of 54 subjects (52%) were successfully linked to care and completed treatment; all had undetectable HCV RNA at end of treatment. Of the 10 subjects tested 12 weeks after completion of treatment, all had undetectable HCV RNA. One infant had detectable HCV RNA (1/23 - 4%).

Conclusion: Co-localization results in increased rates of linkage to care and successful treatment while the observed 4% vertical transmission rate is within previously described rates.

Intracellular Sofosbuvir Concentrations in Pregnant Women With Hepatitis C Virus

Catherine A. Chappell, Kristina M. Brooks, Jennifer K. Kiss, Ingrid S. Macio, Leslie A. Meyn, Kyung-moon Kwon, Cathleen Letterio, Sarjita Naik, Bruce Kreter, Sharon L. Hillier

1University of Pittsburgh, Pittsburgh, PA, USA, 2University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 3Magee—Women's Research Institute, Pittsburgh, PA, USA, 4Gilead Sciences, Inc, Foster City, CA, USA.

Background: Treatment of hepatitis C virus (HCV) during pregnancy could cure maternal HCV during antenatal care engagement and prevent perinatal HCV transmission. We previously showed plasma sofosbuvir (SOF) exposures were 38% higher during pregnancy, whereas the inactive metabolite of SOF (007) was 38% lower compared to non-pregnant women. SOF is converted into its active form, 007-triphosphate (007-TP), within cells and can be used as a surrogate of activation in other tissue types and adherence markers. Our
objectives were to describe 007-TP concentrations in dried blood spots (DBS) and peripheral blood mononuclear cells (PBMCs) during pregnancy.

**Methods:** In this open-label, phase 1 study, HIV-negative pregnant women with chronic HCV infection were enrolled between 23-25 weeks’ gestation and were treated with SOF 400mg/velpatasvir (VEL) 100mg daily for 12 weeks. DBS and PBMCs were collected pre-dose at 3, 6, and 9 weeks of treatment. 007-TP in DBS (17x7mm punch) and PBMCs normalized to 10⁶ cells were measured using validated LC/MS-MS methods. Results were summarized using descriptive statistics. Comparisons to 007-TP concentrations measured in non-pregnant persons with HCV and adherence monitoring were also performed (NCT02573376).

**Results:** Data were available in 10 pregnant people (9 white, 1 Black; median (range) age 31 (25-29) years and weight 75.5 (64.6-102.3) kg). Median (range) serum creatinine, glomerular filtration rate, and hematocrit were 0.5 (0.4-0.6) mg/dL, 126.1 (122.3-140.8) mL/min/1.73 m², and 36.1% (33.2-37.8%), respectively. Geometric mean (%CV) 007-TP in DBS were 340 (287, 403), 340 (278, 418), and 356 (275, 461) fmol/punch at weeks 3, 6, and 9 (A). Geometric mean (%CV) 007-TP in PBMCs were 2111 (1096, 4066), 2808 (1559, 5058), and 2212 (1267, 3864) fmol/10⁶ cells at weeks 3, 6, and 9 (B). In comparison to non-pregnant adults (n=58), 007-TP concentrations in PBMCs were comparable or higher, whereas 007-TP in DBS were ~50% lower in pregnancy.

**Conclusion:** Though 007-TP concentrations were altered in pregnancy, the efficacy of SOF/VEL in this trial was unaffected (100% of those that attended the final maternal visit were cured (n=8)). The decreased concentrations in DBS could be due in part to physiologic hemodilution of pregnancy. Thus, if DBS samples were used to measure adherence in trials, they would need to be adjusted. An international, multicenter trial evaluating the safety and efficacy of SOF/VEL treatment in pregnancy is underway (NCT05140941).

**711 Transfer of Sofosbuvir/Velpatasvir Into Breast Milk**

**Catherine A. Chappell**, Kristina M. Brooks, Jennifer J. Kiser, Brandon Klein, David Nerguizian, Lane Bushman, Hollis L. Laird, Ellen Stewart, Kate Lynska, Kyung-min Kwam, Cathleen Letteris, Bruce Kreter, Elizabeth E. Kranis, University of Pittsburgh, Pittsburgh, PA, USA; University of Colorado Anschutz Medical Campus, Aurora, CO, USA, Mage-Women’s Research Institute, Pittsburgh, PA, USA, Gilead Sciences, Inc, Foster City, CA, USA

**Background:** Treatment of hepatitis C Virus in the postpartum period is delayed until after the completion of breastfeeding due to lack of data on infant exposure and safety. Our objectives were to describe breast milk transfer of sofosbuvir (SOF), GS-331007 (SOF inactive metabolite) and velpatasvir (VEL) in postpartum women being treated for HCV with SOF/VEL who were not intending to breastfeed.

**Methods:** Women undergoing postpartum HCV treatment with SOF 400mg/VEL 100mg daily for 12 weeks and willing to provide pumped breast milk were eligible for the study. SOF/VEL treatment was started within 36 hours of delivery. Paired maternal blood and breast milk were obtained at 2 or more pharmacokinetic (PK) visits. VEL, SOF and GS-331007 concentrations in plasma and breast milk were measured using validated UPLC-MS/MS assays (LLOQ 5 ng/mL for VEL in plasma and breast milk and SOF/007 in plasma; 1 ng/mL for SOF/007 in breast milk). The infant daily dose was calculated using drug concentrations in the milk and considering that the amount of milk ingested by an exclusively breastfed infant is 150 mL/kg/day and reported relative to the adult dose normalized to 70 kg weight and the child dose normalized to 13 kg weight. Data were summarized using descriptive statistics.

**Results:** Four participants were enrolled with a median (range) age of 29.5 years (27-36); 3 white, 1 multiple races; all were multiparous with median (range) weight of 69 kg (58-88) and median creatinine of 0.5mg/dL range (0.5-0.6). The participants had their first PK visit within 24 hours of initiating SOF/VEL and had to 7 PK visits each yielding a total of 17 paired samples for analysis occurring a median (range) time of 1.99 (0.19-6.13) after treatment start. VEL and GS-331007 were quantifiable in all paired samples; SOF was quantifiable in 9 breast milk and 7 plasma samples due to its short half-life (Table). SOF, GS-331007, and VEL concentrations in the breast milk were lower than maternal plasma concentrations. The estimated infant daily dose from breastmilk is less than 0.7% of the daily dose in an adult and a child adjusted weight.

**Conclusion:** Based on our findings, exposure to SOF/VEL via breastmilk is minimal and reassuring for infant safety and low potential for development of HCV resistance with perinatal transmission. Clinicians should consider expedited HCV treatment in the postpartum period during breastfeeding to improve linkage to HCV care.

**Table: SOF, GS-331007, and VEL concentrations in mother and breastmilk**

<table>
<thead>
<tr>
<th>Drug</th>
<th>материнский концентрации (нмоль/л)</th>
<th>Материнский концентрации (нмоль/л)</th>
<th>Материнский концентрации (нмоль/л)</th>
<th>Материнский концентрации (нмоль/л)</th>
<th>Материнский концентрации (нмоль/л)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Софосбувир (SOF)</td>
<td>11.1 (1.3, 68.1)</td>
<td>104.1 (22.4, 237.5)</td>
<td>0.11 (0.84, 1.08)</td>
<td>0.007 (0.002, 0.007)</td>
<td>0.029% (0.003, 0.059)</td>
</tr>
<tr>
<td>GS-331007</td>
<td>2.4 (0.8, 9.5)</td>
<td>161.8 (98.1, 235.6)</td>
<td>0.003 (0.001, 0.002)</td>
<td>0.006 (0.001, 0.003)</td>
<td>0.33% (0.05, 0.20%)</td>
</tr>
<tr>
<td>Вельпатасвир (VEL)</td>
<td>0.1 (0.1, 0.2)</td>
<td>14.0 (4.8, 25.4)</td>
<td>0.01 (0.00, 0.01)</td>
<td>0.001 (0.000, 0.002)</td>
<td>0.14% (0.06, 0.20%)</td>
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**Validation of a Point-of-Care Test Based on RT-LAMP for HCV Detection by Capillary Sampling**

**Sonia Arca-Lafuente**, Cristina Yépez-Notario, Pablo Cea-Callejo, Violeta Lara Aguilar, Celia Crespo-Bermejo, Luz Martín-Carboneros, Ignacio De los Santos, Verónica Briz, Ricardo Madrid, Institute of Health Carlos III, Madrid, Spain; Universidad Pablo de Olavide, Sevilla, Spain; Complutense University of Madrid, Madrid, Spain; La Paz University Hospital, Madrid, Spain; Hospital Universitario de La Princesa, Madrid, Spain

**Background:** Every year, 1.5 million people suffer a new infection by HCV, and nearly 58 million people worldwide live with a Hepatitis C chronic infection. To overcome the underdiagnosis of HCV among hard-to-reach populations and to accomplish WHO’s target of Hepatitis C virus (HCV) elimination by 2030, it is essential to develop new rapid and easy-to-use molecular diagnostic systems with similar performance values to the gold standard qRT-PCR. Loop-Mediated isothermal Amplification (LAMP) assay is a promising tool for point-of-care (POC) molecular detection of viral diseases. In this work, we have validated a new diagnostic tool based on fluorescent reverse transcriptase (RT) LAMP technique, detecting HCV RNA directly from capillary blood samples in less than 50 minutes.

**Methods:** 125 samples from HCV infected patients (116 serum samples [genotypes 1 to 4] and 9 fresh blood samples), 27 individuals who tested negative for HCV but positive for HIV, and 11 healthy controls were analysed. Serum was collected from 50 µL of blood samples by 5 min centrifugation, incubated in 1/300 volume of buffer for 10 min, and then mixed with RT-LAMP reagents. RT-LAMP reactions were run in parallel with two different primer sets: HCG124, a set of 9 primers designed to detect HCV genotypes 1, 2, or 4; and HCDN-G3, a set of 6 specific primers for genotype 3. Reaction mix was first incubated at 43°C for 10 minutes for reverse transcription, and shifted to 68°C for 40 minutes for LAMP reaction. SyBr-green fluorescence signal was recorded in real time.

**Results:** HCG124 and HCDN-G3 primer sets detected HCV RNA in 109/116 HCV positive serum samples and in 8/9 HCV positive blood samples in less than 40 minutes. This new RT-LAMP system has a sensitivity level of 94% and 100% specificity. The limit of detection of the present system is 550 - 1000 IU/mL, lower than WHO recommended limit for HCV POC testing (3000 IU/mL). Real time measurements could be substituted by a single end-point measurement at 40 minutes using a portable fluorescent reader at POC.

**Conclusion:** RT-LAMP system has shown to be highly sensitive and specific for molecular detection of HCV from less than 50 µL blood sample, easily collected by capillary puncture and without a prior RNA purification step. Within less than 50 minutes, an active HCV infection can be detected at POC, implementing
Prevalence and Resistance Profiles of “Unusual” HCV Subtypes in Italy

Collins Ambe Chenwi1, Veliya Chia Di Maio1, Mohammad Al Khathib1, Elisabetta Testi1, Ada Bertelli1, Stefania Paolucci1, Nicola Copolla1, Teresa Pollicino1, Bianca Bruzzone1, Omar El Khathi1, Saba Zaheer Khan1, Massimo Pueti1, Maurizio Zazzi1, Francesca Ceccherini-Silberstein1, for the HCV Virology Italian Resistance Network Vironet C1

1 University of Rome Tor Vergata, Rome, Italy, 2 Istituto di Ricerca sul Cancro, Genova, Genoa, Genoa, Italy, 3 Università di Messina, Messina, Italy, 4 Istituto Superiore di Sanità, Rome, Italy, 5 Università di Siena, Siena, Italy

Background: Recent data show that “unusual” hepatitis C virus (HCV) subtypes have lower responses to direct-acting antivirals (DAAs) compared to most prevalent subtypes. We aimed to investigate the prevalence and resistance profiles of “unusual” HCV subtypes in Italy.

Methods: Clinical and virological data of “unusual” HCV genotype (GT)/subtypes (defined as GT1 non-1a/b, GT2 non-2a/b, GT3 non-3a, GT4 non-4a/d) were analyzed within the Italian VIRONET-C database. Subtype assignment was confirmed by phylogenetic analyses on NS3±NS5A±NS5B sequences. The prevalence of resistance associated substitutions (RASs) was evaluated according to Sorbo et al 2018 list.

Results: Out of 3554 individuals with an available NS3±NS5A±NS5B sequence, 282 (8%) median age [IQR], 73 (57-81) years; 58% males) were infected with “unusual” subtypes (GT1f/g/i/j=3/1/2, GT2c/d/j=24/172, GT3b/h/k=l=1/5/81; GT4c/i/l/m/p/q/r/t/v/=1/1/1/3/5/3/1). Substance use data varied according to ethnicity, with GT2c and GT3h most prevalent in Italians (88-100%), other “unusual” GT3 in Asians (75%), and “unusual” GT1 and GT4 in Africans (78%). 206 individuals (73%) were DAA-naive, while 76 were DAA-failed (27%), in particular 36 with a recommended regimen (22 glecaprevir/pibrentasvir and 14 sofosbuvir/velpatasvir). The patients infected with GT3 unusual subtypes were more prone to failure (91%, 10/11) followed by GT4 (44%, 7/16), GT2 (23%, 58/249) and finally GT1 (17%, 1/6). All failed patients (except one with GT4n) displayed at least one RAS in at least one protein (Figure 1). The number of RASs in each protein varied by GT, subtype and treatment exposure. Overall, NS5A-RASs were most prevalent, with complex patterns in both DAA-naive and failures, NS3-RASs were less prevalent but present in all GT failures, while NS5B-RASs were rare and present across GT1-3, GT4, with variable patterns. NS3A-RASs at position 93 (NF/V/S) were detected only at failure in GT3h/k and GT4o/v. Few patients had NS3-RASs, only at failure (3/3 GT3h:80R or 158V; 4/24 GT2c:56Y/H=168V/A).

Conclusion: In this Italian setting, GT1-4 unusual subtypes were frequent, predominated by GT2c in Italians, and with failure rates highest within GT3. Most DAA-failures carried complex NS5A-RAS patterns, some conferring high-level of resistance. These results advise for closer surveillance and further studies to better characterize the impact of “unusual” HCV subtypes on DAA efficacy.

Simultaneous Start Strategy With BIC/FTC/TAF in Individuals With HIV/HCV Coinfection in China

Qing Lin1,2, Feng Wang3,4, Li Lü5,6, Hongmei Zhu2, Yuan Yao7,8, Jingchun He9, Zheng Li2, Zhenglin Wang1, Qingmei Zhou1,2, Zehu Deng9,11, Francisco Tellez1, Hongmei Zhu9, Teresa Pollicino2, Li Li1, for the HCV Virology Italian Resistance Network Vironet C1

1 ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, 2 IRCCS Policlinico San Matteo Foundation, Pavia, Italy, 3 University of Campania Luigi Vanvitelli, Naples, Italy, 4 University of Messina, Messina, Italy, 5 IRCSS IRCCS Don Carlo Gnocchi Ospedale Maggiore Policlinico, Milan, Italy, 6 University of Siena, Siena, Italy

Background: Highly Active Antiretroviral treatment (ART) with Direct-acting antiviral agents (DAAs) has demonstrated high efficacy and favorable safety profile in HIV/HCV co-infected individuals with HIV viral suppression and stable immune status. However there is great need in rapid initiation of ART and anti-HCV treatment regardless of HIV viral suppression status and CD4 T cell count as a simplified treatment strategy-fo “Simultaneous Start” treatment for both HIV and HCV.

Methods: We conducted a retrospective, single-center study in Southwest China from May 2021 to August 2023. The study aimed to evaluate effectiveness and safety of immediate initiation or switch to bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF) with sofosbuvir/velpatasvir (SOF/VEL) administered once daily in PWIH (people with HIV with HCV) with recent acquired HCV. The primary endpoints were HIV and HCV viral suppression rate, defined as HIV RNA <20 cp/mL at 12 weeks and HCV RNA <15 IU/mL at 24 weeks after the end of HCV treatment (SVR24).

Results: Of the 128 patients enrolled, mean age of 36 years (31-39, IQR), 77% male (n=99), 52 were ART naive (TN), 76 were ART experienced (TE). The baseline characteristics were summarized in Table 1. All patients achieved HIV RNA suppression at 12 weeks and 36 weeks, 98.4% (126/128) patients achieved HCV SVR24. Among individuals with baseline CD4 T cell count < 200 cells/mm3 (n=32, 25%), all achieved SVR 24. And 98% (94/96) of those with baseline CD4 cell count > 200 cells/mm3 (n=96, 75%) achieved SVR 24. The two patients who did not achieve SVR24 were PWIH (people who inject drugs) infected with HCV genotype 3, had cirrhosis and poor adherence. Nine HBV/HCV co-infected patients maintained or achieved HBV DNA undetectable at 12 weeks and 36 weeks of treatment. No patient discontinued treatment due to adverse events. Conclusion: Immediate initiation or switch to BIC/FTC/TAF with SOF/VEL treatment provided high HIV and HCV suppression rate with a favorable safety profile. The study suggests that HCV treatment can start immediately without waiting for the CD4 T cell count to exceed 200 cells/mm3 in HIV/HCV co-infected individuals. This simplified “Simultaneous Start” treatment may be a feasible and easy treatment strategy for HIV/HCV co-infected individuals. The figure, table, or graphic for this abstract has been removed.
HIV/HCV: 90% and 87% (p = 0.707). There was no difference in hepatic and non-hepatic mortality between the two subpopulations. In multivariable analysis adjusted for age, sex, HIV infection and PS, independent predictors of all-cause death were age, MELD index and LSM at the time of SVR, but not HIV infection (HR [95% CI]: 1.01 [0.67–1.53]; p = 0.970).

Conclusion: In patients with HCV infection, HIV coinfection does not reduce overall survival or mortality attributable to hepatic and non-hepatic causes after SVR. Differences in the control of HIV infection or in other survival-limiting factors could explain the disparity in the data found in the different cohorts.

716 Direct-Acting Antivirals and Risk of Hepatocellular Carcinoma in People With HIV/HCV Coinfection

Daniela K. van Santen, for the HepCausal Collaboration
Harvard TH Chan School of Public Health, Boston, MA, USA

Background: Hepatitis C virus (HCV) infection is a leading cause of hepatocellular carcinoma (HCC). While direct-acting antivirals (DAAs) cure ~95% of individuals with HCV, precise estimates of HCC risk and of risk trends post-DAA are urgently needed to inform HCC surveillance. We aimed to estimate the effect of DAA treatment on HCC risk in people with HIV/HCV co-infection and advanced liver fibrosis or cirrhosis.

Methods: We emulated a target trial using data on individuals with HIV/HCV from HepCausal, a collaboration of 12 cohorts from Europe and North America. The eligibility criteria were: HIV infection, chronic HCV infection, advanced liver fibrosis or cirrhosis (clinical cirrhosis diagnosis, liver stiffness ≥ 9.5 kPa, or FIB-4 ≥ 2.5), DAA naive, HIV-RNA < 200 copies/mL, on antiretroviral therapy, no hepatitis B virus co-infection, and no previous HCC diagnosis or liver transplant. The treatment strategies were DAA initiation versus no DAA use during the follow-up. The outcome was incident HCC. We emulated a sequence of trials starting each month between Jan 2013 and Dec 2022 and individuals could be eligible in and contribute follow-up to multiple trials. We censored individuals in the no DAA group if they initiated DAA. We estimated the 4-year cumulative incidence of HCC under the two DAA strategies with adjustment for confounding and loss to follow-up via inverse probability weighting.

Results: Of 3,871 eligible individuals (87% males, median age of 56 [IQR: 50, 61]), 71% initiated DAA and 108 had an incident HCC diagnosis. These individuals contributed on average to 19 trials. The estimated 4-year cumulative incidence of HCC among the two DAA strategies with adjustment for confounding and loss to follow-up was 2.9% (95% CI, 1.8–4.2) and 4.4% (95% CI, 2.9–6.2), respectively.

Conclusion: We estimated that DAA reduces the 4-year HCC risk by 2% on an absolute scale and 55% on a relative scale in people with HIV/HCV co-infection and advanced fibrosis or cirrhosis. The annual HCC risk after DAA initiation was close to the threshold (0.4%) beyond which HCC surveillance is deemed cost-effective, which suggests that continued post-cure HCC monitoring is warranted in this population.

717 Predictors of Liver-Related Events Following DAA HCV Cure in PWH With Advanced Fibrosis/Cirrhosis

Juan Berenguer, Teresa Aldámez-Echevarría, Victor Hontañón, Chiara Fanciulli, Carmen Quereda, Carmen Busca, Lourdes Dominguez, Cristina Hernández, Jorge Vergas, Lucio J. García-Fraile, Marta De Miguel, Cristina Díez, José M. Bellón, Juan González-García, for the GeSIDA MARATHON Study Team
1 Hospital General Universitario Gregorio Marañón, Madrid, Spain, 2 La Paz University Hospital, Madrid, Spain, 3 Hospital Ramón y Cajal, Madrid, Spain, 4 Hospital Universitario de La Princesa, Madrid, Spain, 5 Hospital Universitario de La Princesa, Madrid, Spain, 6 Hospital Universitario Clínicas, Madrid, Spain

Background: We assessed prognostic factors of liver-related events (LRE) among HCV-coinfected PWH with advanced fibrosis (AF) or compensated cirrhosis (CC) with follow-up after all-oral direct antiviral therapy (DAA-Rx).

Methods: We leveraged 3 prospective observational studies in Spain to select coinfected PWH with AF (biopsy confirmed F3 or liver stiffness [LS] value ≥ 9.9 kPa and ≤ 12.5 kPa) or CC (biopsy confirmed or LS > 12.5 kPa) with SVR following DAA-Rx from 2014 to 2017. The primary outcome was an LRE: decompensation (DC) or hepatocellular carcinoma (HCC), whichever occurred first after the finalization of DAA therapy. Independent variables (based on the underlying conceptual framework) included liver disease category, age, sex, smoking, alcohol abuse, methadone use, prior clinical AIDS, CD4+ T-cell count, albumin concentration, metabolic syndrome, TyG and HSI indexes, and values of LS and FIB4 at baseline and 1 year after finalization of DAA therapy (1-yr). Multivariable competing-risk regression analyses were performed by the Cox proportional hazards model. Outcomes generated by the model were evaluated using time-dependent AUCs.

Results: Of 1,145 PWH (384 AF and 761 CC) were included. After a median follow-up of 41 months, 60 patients died, 24 had DC, and 21 developed HCC. The risk of LRE was higher among those with CC than those with AF, but no statistically significant differences in overall and cause-specific mortality were found between groups. Albumin concentration (aSHR [95% CI]: 0.55 [0.35 – 0.87]) per g/L increase and 1-yr LS values (aSHR [95% CI]: 1.04 [1.01 – 1.09] per kPa increase) were the only factors independently associated with the risk of LRE. The best cutoff value of 1-yr LS to predict LRE was 12.5 kPa (NPV: 99.5% [95% CI: 98.2 – 99.9]). For each 5 kPa increase above this cutoff, the HR of LRE was 1.34 (95% CI, 1.24 – 1.45). When we took the 12.5 kPa cutoff as the reference, the HR of LRE was 1.34 (95% CI, 1.24 – 1.45).

Conclusion: Albumin concentration and 1-yr LS values after the finalization of a successful DAA therapy were identified as independent predictors of LRE among HCV-coinfected PWH with AF or CC. The best cutoff value of 1-yr LS to predict LRE was 12.5 kPa.
718 Immunophenotypic Profile Modifications After Spontaneous HCV Clearance or DAA Treatment in PWHIV
Camille Jacqueline1, Violeta Lara Aguilar1, Manuel Llamas-Adán1, Cristina González Díaz1, Sergio Grande-García1, Celia Crespo-Bermejo1, Sonia Arca-Lafuente1, Luz Martín-Carbonero1, Pablo Ryan2, Ignacio De los Santos2, Verónica Birl2, Amanda Fernández-Rodríguez2
1Institute of Health Carlos III, Madrid, Spain, 2Hospital La Paz Institute for Health Research, Madrid, Spain
Background: Acquisition of HCV is frequent among people with HIV (PWHIV), an event that profoundly modifies the natural history of both infections. Our goal was to analyze whether HCV clearance by direct-acting antivirals (DAAs) after chronic infection can partially restore both molecular and cellular immune profile to levels similar to those found in PWHIV. In addition, we explored whether changes over time in these patients were similar to those found in patients who cleared HCV spontaneously after acute infection.
Methods: We performed a longitudinal observational study in 88 PWHIV: i) 31 with active chronic HCV infection (CHC) prior to starting DAA therapy and 48 weeks after achieving sustained vireological response (SVR); ii) 25 who previously cleared HCV spontaneously (AE); iii) 32 controls (HIV) never infected with HCV. Both AE and HIV also were follow-up 48 weeks after first sample. A total of 27 CD4+ and CD8+ peripheral T cell subsets were assessed by spectral flow cytometry (18 antibodies) as well as 73 plasma molecules by multiplex immunoassay (Luminex, procartaplex). Differences in cell populations and plasma molecules were assessed by generalized linear models adjusted for the most significant clinical variables and multiple comparisons (p-value corrected by Benjamini-Hochberg q<0.15).
Results: At follow-up, lower levels of senescence markers (PD1, CD57) and central memory (CD3+CD4+ or CD6+CD45RA-CCR7+CD27+CD28+) and naïve (CD3+CD4+ or CD8+CD45RA-CCR7+CD27+CD28+) CD4+ and CD8+ T cells were observed in the CHC group compared to control. On the other hand, the AE group showed an increase in exhausted immune cells and immunosenescence markers over time to levels similar to those of HIV+ controls patients. Also, AE group showed a higher level of effector memory CD4+ and CD8+ T cells (EM:CD45RA-CCR7-; EM TH01: CD45RA-CCR7-CD28+CD27+; EM TH1: CD45RA-CCR7-CD27-; EM TH2: CD45RA-CCR7-CD28+CD27-; EM TH12: CD45RA-CCR7-CD28+CD27-) than HIV. The immune profile of the CHC and AE groups were very similar at follow-up. Regarding plasma molecules, 48 weeks after achieving SVR, a decrease in proinflammatory cytokines, checkpoint inhibitors and immune cell activation was observed compared to values before initiation of DAAs therapy.
Conclusion: Clearance with DAAs after chronic HCV infection improved cellular senescence profile but remained higher than controls. The slightly higher levels of circulating immunosenescence markers after 1-year follow-up in the AE group may indicate possible accelerated aging driven by acute HCV infection.

719 Influence of Markers Related to Biological Aging on Liver Regeneration in HCV Patients Achieving SVR
Alejandro González-Serna1, Anaísa Corpina-Gómez2, Chialaughter Del Prieto1, Mercedes Cano1, Marta Santos1, Carmen Martín-Sierra1, Pilar Rincón1, Juan Antonio Pineda2, Luis M Real3, Juan Macías3
1University of Sevilla, Sevilla, Spain, 2Hospital Universitario de Valme, Sevilla, Spain, 3University of Sevilla, Sevilla, Spain
Background: The achievement of sustained viral response (SVR) is associated with a reduction in HR in most patients and therefore, with a lower risk of suffering hepatic events. However, in a non-negligible percentage (10-30%) of patients who achieve SVR, liver stiffness (LS) is not reduced, and the responsible factors are not yet known. The analysis of markers related to cellular aging could offer an answer to this question.
Methods: Prospective study of parallel GEHEP and HEPAPVR cohorts at the Virgen de Valme University Hospital that met the following criteria: 1) SVR after 12 weeks with interferon-free direct-acting antivirals (DAAs); 2) HR > 9.5 kPa 3 months before treatment; 3) LS measurement and sample available on the SVR evaluation date. We will measure telomere length (RTL) and the levels of mitochondria, hsCRP, sCD163, sCD14, IL-6, Isoprostanes-8, CXC10, CCL11, VWF, Vitamin D and CHI3L1, and we will analyze their association with the decrease or not of the LS at RVS.
Results: A total of 175 patients were included. In 31 (18%) patients the LS did not decrease, going from a median (Q1-Q3) of 14.4 (10.3-27.7) kPa at the beginning of treatment to 21.3 (14.4-46.4) kPa in SVR. On the other hand, the group of 144 (82%) patients in whom LS decreased went from a median of 16.8 (11.9-28.6) kPa at the beginning of treatment to 11.5 (7.6-1.8) kPa in SVR. In the univariate analysis, the only variables that showed an association with the absence of LS decrease in SVR were RTL (8.06 (5.83), 11.09) vs 6.41 (4.78, 8.29) [p=0.011], hsCRP [0.8 (0.35, 1.91) vs 1.55 (0.63, 4.24) (p=0.008)], and sCD163 [49.3 (39.8, 58.4) vs 45.2 (40, 47.9) (p=0.045)]. Factors such as HIV-coinfected baseline HR, parenchymal infection, HCV-3 genotype, HCV viral load, or the MELD and Child-Pugh A indices showed no association. In the multivariate analysis adjusted for sex, age, and hsCRP and sCD163 levels, greater RTL size was the only variable independently associated with the absence of LS decrease (OR 1.73; 95% CI (1.02-1.35); p = 0.025).
Conclusion: Telomere length is independently associated with the absence of LS decrease in SVR. Greater cellular aging could be responsible, at least partially, for the absence of LS decrease in patients who achieve SVR.

720 Hepatitis C Reinfection Among Men Who Have Sex With Men in an Acute HIV Cohort in Thailand
Pathariya Promsena1, Ferron F. Ocampa2, Carlo P. Sacadana1, Sutteaporn Pinyakorn2, Nisakorn Ratnaratorn2, Kulfa Rattanave2, Nitaya Chomchey1, Somchai Siripienchan1, Nittaya Phanuphak1, Sandhya Vasan1, Lydie Traitmann3, Donn J. Golby3 for SEARCH1024
1SEARCH, Bangkok, Thailand, 2US Military HIV Research Program, Silver Spring, MD, USA, 3Institute of HIV Research and Innovation (IHRI), Bangkok, Thailand
Background: The advent of direct-acting antivirals (DAAs) revolutionized the treatment of hepatitis C (HCV). However, HCV reinfections either after spontaneous clearance or sustained virologic response (SVR) pose a challenge in HCV elimination, particularly in high-risk populations. In this study, we
determined the incidence and identified risk factors of HCV reinfection in a longitudinal early-treated acute HIV cohort in Thailand.

**Methods:** SEARCH010/RV254 enrolls participants during acute HIV infection (Fiebig I-IV) and initiates antiretroviral therapy (ART) within days of diagnosis. HCV antibody screening was performed at enrolment and then annually or if clinically indicated. HCV infection was confirmed with HCV ribonucleic acid (RNA). SVR was assessed at least 12 weeks after treatment completion. HCV RNA was monitored annually in all participants with HCV clearance (either spontaneous clearance or SVR). HCV reinfection was defined as positive HCV RNA after HCV clearance. Incidence of HCV reinfection with 95% confidence intervals (CI) per 100 person-years of follow-up (PYFU) was calculated using the exact method. Cox regression was used to identify risk factors for HCV reinfection.

**Results:** Between May 2009 and August 2023, 127/724 (17.5%) participants were diagnosed with HCV infection. Data analysis included 91 participants who had spontaneously cleared (n=18) or achieved SVR after HCV treatment (n=73). Among these, 14 (15.4%) developed reinfection; five after spontaneous clearance and nine after SVR. All were men who have sex with men (MSM) with median age 34.0 (IQR 29.0-37.0) years. The median time to reinfection was 1.3 years (IQR 0.8-2.8). The overall HCV reinfection incidence density rate was 6.3 per 100 PYFU (95% CI, 3.7-10.6) over 222.8 PYFU. HCV reinfection incidence was higher in the spontaneous clearance group (9.4 per 100 PYFU, 95% CI, 3.9-22.6) than the SVR group (5.3 per 100 PYFU, 95% CI, 2.8-10.2), P=0.283. Although a high proportion of participants reported current illicit drug use (50.5%), current injection drug use (33.6%), condomless sex (37.4%), or had syphilis within the previous 6 months (26.4%), no significant associations between these risk factors and HCV reinfection were found in univariable analyses.

**Conclusion:** In this early-treated AHI cohort of Thai MSM, there is a high incidence of HCV reinfection. Post-clearance follow-up with counseling and preventive strategies to reduce ongoing risk behavior are needed to reduce HCV reinfection. The figure, table, or graphic for this abstract has been removed.

**722 Re-Infection Following a Minimal Monitoring Approach for Treatment of Hepatitis C Virus Infection**

Win Min Han1, Saniul Sabas Solomon1, Laura Smeton3, Sandra Wagner-Cardoso2, Jiani Li1, P.C. Parvangada2, Mark Sulkowski2, Susanna Nagogie2, Ross Martin2, Hongmei Mo3, Evgenia Maiorova2, David L. Wyles1

1HIV-NAT, Bangkok, Thailand, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 3HIV/AIDS Channing School of Public Health, Boston, MA, USA, Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil, "Gilead Sciences, Inc, Foster City, CA, USA, "Duke University, Durham, NC, USA, "Dover Health Medical Center, Denver, CO, USA

**Background:** MINMON (ACTG A5360) trial demonstrated that a minimal monitoring approach for HCV treatment with sofosbuvir-velpatasvir (SOF/VEL) was safe and efficacious (sustained viral response [SVR] 95%) in a diverse global population. In a phylogenetic analysis, we estimated HCV re-infection rates among participants post-treatment HCV RNA >lower limit of quantification (LLOQ).

**Methods:** HCV RNA evaluations were scheduled at weeks 0, 24 (SVR visit), 48 and 72 for MINMON participants. Samples with post-baseline HCV RNA >LLOQ and paired baseline samples underwent deep sequencing of NSSA and NSSB genes. Consensus sequences determined HCV genotype. FastTree generated phylogenetic trees; FigTree provided visualization. Participants whose post-treatment HCV sequence was different from baseline were defined as re-infection. Intermingling of sequences represented treatment failure. Re-infection rates were calculated using person-time of observation starting at the final reported date of SOF/VEL and ending at the earlier of HCV RNA >LLOQ or final study visit. Re-infection rates per 100 person-years (PY) were calculated with 95% confidence intervals constructed using Poisson distribution.

**Results:** SVR in the primary analysis (intention to treat [ITT]) was 95% [95% CI 92.4-96.7] (379/399). Of 397 participants who had post-entry HCV RNA, 29 participants had HCV RNA >LLOQ and sequencing data available for re-infection analysis. Of these, six participants initially designated as non-SVR, and 11 participants initially classified as cured were determined to be HCV re-infections by phylogenetic analysis (total 17 re-infections). The SVR adjusting for re-infection was 96.5% [95% CI 94.2-97.9] (385/399) compared to the ITT SVR of 95%. Of all participants, 17 had re-infection during 438 person-years of follow-up (re-infection rate 3.9 per 100 PY [95% CI 2.4-6.2]). Of them, 12 were from HCV RNA collected at week 48 or 72. All 17 participants with HCV re-infection were assigned male sex at birth (13 endorsing MSM), 15 living with HIV, 14 had baseline genotype 1a, and 13 were from Thailand (of whom 11 were MSM with HIV and genotype 1a).

**Conclusion:** Discounting re-infections, SVR observed in MINMON was 96.5%. The high HCV reinfecation rate even prior to SVR, especially among MSM living with HIV underscores the need to scale-up evidence-based interventions to reduce re-infection while simultaneously increasing screening and treatment to minimize HCV viremic burden in the population.

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**723 Impact of Healthcare Expenditure on HBV and HCV in Southern Western European Countries in 2000-2019**

Claudia Palladino1, Rebecca Rams1, Hafenyi J. Ezeonwumelu1, Nuno Taveira2, Verónica Briz1

1Universidade de Lisboa, Lisbon, Portugal, 2Institute of Health Carlos III, Madrid, Spain, 3Gladelstone Institute of Virology and Immunology, San Francisco, CA, USA

**Background:** Viral hepatitis remains a threat to public health. This Global Burden Disease study was conducted to investigate the impact of the economic crisis that began in 2008 and which severely affected the Southern Western European (SWE) countries (Greece, Italy, Portugal, Spain), on the burden of HBV and HCV disease.

**Methods:** Time series modelling was performed to quantify the impact of healthcare expenditure, defined as a percentage of gross domestic product, on the trend of the epidemiological estimates for HBV and HCV over the period 2000-2019. Estimates were retrieved from the Global Health Data Exchange of the Global Burden of Diseases 2019.

**Results:** Mortality rates due to HBV stabilised in SWE during the period 2000-2019. Declining trends in incidence and prevalence of acute HBV and chronic HBV were observed in SWE, although the pace of decline was slower in the period 2010-2019. Acute HCV metrics and chronic HCV incidence and mortality showed a stable trend in SWE, whereas the prevalence of chronic HCV showed a fluctuating trend. Liver cancer due to both hepatitis infections showed a stagnating burden over time. An inverse association was observed between healthcare expenditure and both acute HBV and HCV infections, with results close to significance for acute HBV disability-adjusted life years (DALYs) [-2.54 (-5.09 - 0.01); p=0.05] and years of life lost to premature mortality (YLLs) [-2.53 (-5.09 - 0.03); p=0.05] in Greece. For acute HCV, the results showed that one unit (percentage) increase in healthcare expenditure was associated with a more beneficial effect on reducing incidence cases in Italy [-8.93 (-34.31 – 16.46); p=0.49], up to 80 times higher than in the other SWE countries. A similar inverse association between healthcare expenditure and cirrhosis and other chronic liver diseases (CCLD) metrics was found for both HBV and HCV, except for CCLD-HBV prevalence in Portugal and Spain. In addition, a positive but not significant association was found between healthcare expenditure and liver cancer metrics for both HBV and HCV.

**Conclusion:** Epidemiological indicators for HBV and HCV showed a slower pace of decline in the period 2010-2019, with a better improvement for HBV and a stabilization of mortality and liver cancer burden due to both hepatitis. The economic crisis of 2008 had a negative impact on the burden of hepatitis B and C. Elimination of HBV and HCV by 2030 will be a major challenge in the SWE countries.

**724 Epidemiologic Burden of Hepatitis D Virus in the United States**

Elizabeth M. Marlowe1, Brooke E. Swanson, Susan E. Realegeno, Ron M. Kagan, William A. Meyer

1Quest Diagnostics, San Clemente, CA, USA

**Background:** Hepatitis D affects nearly 5% of people globally who have a chronic infection with hepatitis B, according to the World Health Organization. The prevalence of hepatitis D virus (HDV) in the United States is generally considered lower than in other countries; however, HDV seroprevalence studies of the US population are limited, and reported ranges vary depending on the study population. To increase HDV detection, universal HDV testing of hepatitis B surface antigen (HBsAg)-positive specimens has been proposed. The objective of this study was to estimate the epidemiological prevalence of HDV infection within the United States in HBsAg-positive specimens.

**Methods:** Consecutive unique deidentified remnant HBsAg-positive specimens submitted from August to September 2023 for routine clinical testing at Quest Diagnostics, representing each of the ten Health and Human Services (HHS) regions in the United States, were included in the study. A reflex algorithm using HBsAg-positive specimens for HDV total antibody testing was utilized.
and further testing of anti-HDV antibody positive-specimens for HDV RNA was conducted.

**Results:** A total of 2,379 HBsAg-positive specimens were included in the study. The overall cohort was 46% female with a mean age of 50.1 years (50.8 years male, 49.4 years female). The overall seroprevalence of anti-HDV was 1.8%. The highest number of anti-HDV-positive specimens (n=8) were observed in HHS regions 3, 4, and 9, with prevalence (95% CI) of 0.031 (0.010-0.052), 0.017 (0.005-0.028), and 0.018 (0.006-0.030), respectively. Of the 42 anti-HDV-positive specimens, 31% were also positive for HDV RNA (viral load range: 198-1,070,000 IU/mL; n=10; <40 IU/mL; n=3).

**Conclusion:** These data suggest that HDV infection prevalence is relatively low in the United States. Further HDV seroprevalence studies are needed to determine which specific US regions would obtain the greatest benefit from future potential HDV-related interventions.

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**725 Incidence and Outcome of HDV Infection in People With HIV in the Era of Tenofovir-Containing Therapy**


National Taiwan University Hospital, Taipei, Taiwan

**Background:** Tenofovir-containing antiretroviral therapy (ART) achieves high rates of HBV suppression and improves survival among HBV-infected people with HIV (PWH). We investigated the incidence of HDV infection among HBV-infected PWH and the impact of HDV on mortality and liver-related outcomes in the era of tenofovir-containing ART.

**Methods:** HBV-infected PWH seeking HIV care at the National Taiwan University Hospital between 2011 and 2022 were included and followed until September 2023. Screening for anti-HIV antibodies was performed on an annual basis on sequentially archived blood samples collected from the included PWH. The timing of incident HDV infection was estimated by the midpoint between the last time point of blood samples tested HDV-seronegative and the first time point of samples tested HDV-seropositive. Comparisons of clinical outcomes between HBV-infected PWH with and those without HDV infection were analyzed using the Kaplan-Meier method and multivariate Cox proportional hazards models.

**Results:** A total of 534 PWH who had chronic HBV infection were included and 36 (6.7%) tested seropositive for HDV at baseline. The median age of the included PWH was 37.8 years (IQR, 32.6-44.8) and 438 (82.0%) were men who have sex with men. Among the 498 PWH testing HDV-seropositive at baseline, 49 (9.8%) seroconverted for HDV, with 45 who were receiving tenofovir-containing ART at the time of seroconversion. After a total of 3873.54 person-years of follow-up (PYFU), the overall incidence rate of HDV superinfection was 12.65 per 1000 PYFU. After a median follow-up duration of 9.6 years (IQR 5.3-12.2), with 90.3% of the total follow-up time covered by tenofovir-containing ART at the time of seroconversion. After a total of 12,292 patients with HBV/HIV coinfection, we identified 608 patients with HDV and 11,177 with no evidence of HDV. At baseline, individuals with HDV were more likely to have claims for compensated (13.3 vs. 8.4%) and decompensated (10.2 vs. 6.0%) cirrhosis, HCC (1.6 vs. 1.1%), and liver transplant (1.0 vs. 0.3%). Among individuals with no evidence of these conditions at baseline, the risk of developing cirrhosis was 1.20 (0.85 – 1.69) for HDV vs. no HDV and among those with compensated cirrhosis at baseline, the risk of decompensation, HCC, or liver transplant was 1.72 (0.99 – 3.00). A sensitivity analysis excluding individuals with HDV at baseline yielded similar results. HDV prevalence was 6.8% vs. 7.4% (p < 0.01) and the prevalence of injection drug use among individuals with HDV was 12.8% vs. 27.7% (p < 0.01) in the HBV and HBV/HIV cohorts respectively.

**Conclusion:** HDV infection is associated with poor liver health at baseline and increased risk of liver-related events in individuals with HBV/HIV coinfection. HDV and HIV/PWID prevalence was significantly higher among patients with HBV/HIV co-infection than in the general HBV population in this dataset. The figure, table, or graphic for this abstract has been removed.

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**727 Changes in Liver Fibrosis Stage Among People Living With Untreated Hepatitis B in Senegal**

Adrià Ramirez Mena, Laura E. Telep, Grace M. Chee, Amanda W. Singer, Anand P. Chokkalingam, David L. Wyles

Gilead Sciences, Inc, Foster City, CA, USA, Boston Medical Center, Boston, MA, USA

**Background:** Up to 10% of individuals with human immunodeficiency virus (HIV) are coinfected with hepatitis B virus (HBV). Limited data are available describing the natural history of triple infection with HBV/HIV/hepatitis delta virus (HDV). This study examines baseline liver health, HDV prevalence, and the risk of liver-related events in individuals in United States (US) administrative claims data with HBV/HIV with or without HDV.

**Methods:** A retrospective cohort study was conducted in the HealthVerity dataset which includes US medical and pharmacy claims, electronic medical records, and hospital data from years 2015 – 2022 for > 100 million individuals. Those included were ≥18 years at cohort entry with one inpatient or two outpatient international classification of disease (ICD-9/10-CM) codes at least 30 days apart for each type of viral infection. Cohort entry was the date of the last infection among HBV, HIV, and HDV. All included patients had continuous insurance enrollment for 365 days prior and at least one day after cohort entry. We used Cox proportional hazard methods to estimate risk of liver-related events in individuals with vs. without HDV. Prevalence of HDV was assessed using any single HDV claim in any enrollment period.

**Results:** Of the 12,292 patients with HBV/HIV coinfection, we identified 608 patients with HDV and 11,177 with no evidence of HDV. At baseline, individuals with HDV were more likely to have claims for compensated (13.3 vs. 8.4%) and decompensated (10.2 vs. 6.0%) cirrhosis, HCC (1.6 vs. 1.1%), and liver transplant (1.0 vs. 0.3%). Among individuals with no evidence of these conditions at baseline, the risk of developing cirrhosis was 1.20 (0.85 – 1.69) for HDV vs. no HDV and among those with compensated cirrhosis at baseline, the risk of decompensation, HCC, or liver transplant was 1.72 (0.99 – 3.00). A sensitivity analysis excluding individuals with HDV at baseline yielded similar results. HDV prevalence was 6.8% vs. 7.4% (p < 0.01) and the prevalence of injection drug use among individuals with HDV was 12.8% vs. 27.7% (p < 0.01) in the HBV and HBV/HIV cohorts respectively.

**Conclusion:** HDV infection is associated with poor liver health at baseline and increased risk of liver-related events in individuals with HBV/HIV coinfection. HDV and HIV/PWID prevalence was significantly higher among patients with HBV/HIV co-infection than in the general HBV population in this dataset. The figure, table, or graphic for this abstract has been removed.
m², 23.6% had HBV DNA>20,000 IU/ml and 5.2% had ALT>40 IU. Significant fibrosis was present in 25 (4.5%) individuals at baseline and in 36 (6.5%) at 12 months. Overall, 4.5% (25/556) of participants experienced liver fibrosis progression during follow-up, of whom 24 (96.0%) had F0-F1 fibrosis at inclusion. In multivariable analyses, male sex (adjusted odds ratio 2.77, 95% confidence interval 1.05-7.33) was the only characteristic associated with fibrosis progression.

Conclusion: SEN-B is one of the largest prospective cohorts of people with HBV with longitudinal data on liver fibrosis in sub-Saharan Africa. Five percent of individuals ineligible for antiviral therapy experienced a progression of liver fibrosis stage during the first year of follow-up and this outcome was more likely in men than women. Long-term data is urgently needed to understanding the determinants of liver fibrosis changes in Africa in order to inform managing strategies.

728 Prevalence and Incidence of Hepatitis B Among People Living With HIV in 4 Sub-Saharan Countries
Josaphat Rosget1, Nicole Dear1, Hannah Kibuuka1, John Owwooth1, Jonah Maiswai1, Valentine Sing'oei1, Emmanuel Bahemana1, Victor Anyebe2, Debika Bhattacharya2, Georg Laeser3, Arthur Kim4, Trevor A. Crowell4, Neha Shah5, Julie Ake6, for the AFRICOS Study Group
1H&F Medical Research International, Kericho, Kenya, 2US Military HIV Research Program, Bethesda, MD, USA, 3Makerere University, Kampala, Uganda, 4H&F Medical Research International, Kisumu, Kenya, 5H&F Medical Research International, Mbuyi, United Republic of Tanzania, 6Walter Reed Army Institute of Research, Silver Spring, MD, USA, 7University of California Los Angeles, Los Angeles, CA, USA, 8Henry M Jackson Foundation, Bethesda, MD, USA, 9US Military HIV Research Program, Silver Spring, MD, USA

Background: Chronic Hepatitis B is increasing despite it being a vaccine-preventable disease. Africa remains among the regions with the highest number of hepatitis B surface antigen (HBsAg) positive individuals with many studies suggesting HBV transmission occurring predominantly in childhood. To better understand the burden of hepatitis B infection in Sub-Saharan Africa, we assessed the prevalence and incidence of hepatitis B in a prospective cohort study in four African countries.

Methods: The African Cohort Study (AFRICOS) is an ongoing observational cohort that started in 2013. The study enrolls participants from PEPFAR-supported HIV clinical sites across five programs (South Rift Valley, Kenya; Kisumu West, Kenya; Kayunga, Uganda; Mbuya, Tanzania; and Abuja & Lagos, Nigeria) in four countries. People with HIV are enrolled and followed through twice-yearly visits for up to 15 years. Hepatitis B screening is performed at enrollment and annually with HBsAg test, with positive tests confirmed with HBsAg ELISA. Hepatitis B incidence rates and 95% confidence intervals (CIs) were estimated, using a Poisson distribution, as the number of new hepatitis B diagnoses per 1000 person-years (PY) of follow-up.

Results: As of June 2023, 3368 people living with HIV were enrolled, 57.9% being females, with a median age of 41.4 years. At enrollment, 216 participants (6.4%) had a reactive HBsAg result for HBV. Nigeria had the highest prevalence at 14.0% followed by Kisumu West (9.1%), with South Rift Valley having the lowest prevalence at 1.6%. There was a significant difference in prevalence between male and female participants (8.3% v 5.0%; p<0.001). There were 63 incident cases for an incidence rate of 4.4/1000 PY (95% CI: 3.5 – 5.7). The median age for the incident cases was 42.7 years (IQR: 36.4-48.9). Kisumu West had the highest incidence rate (9.2/1000PY, 95% CI: 6.20-14.31) followed by Nigeria (8.60/1000PY, 95% CI: 4.76-15.53), Tanzania (5.61/1000PY, 95% CI: 3.26-9.66) and Uganda (3.41/1000PY, 95% CI: 1.78-6.58). South Rift Valley had the lowest incidence rate at 1.41/1000PY (95% CI: 0.70-2.81).

Conclusion: We observed regional variation in the prevalence of HBV infection. We also demonstrated incident cases of Hepatitis B among adults living with HIV in Sub-Saharan Africa indicating that transmission also occurs after childhood. These findings have implications for frequency of repeat HBsAg screening in PLH in Africa, particularly where HBV vaccination status may be unknown.

729 Loss of Serologic Response in Young People With HIV (PWH) Who Had Undergone HBV Revaccination
Yi-Chia Huang, Hsin-Yun Sun, Sung-Hui Huang, Yu-Chung Chuang, Yu-Shan Huang, Kuan-Yin Lin, Aristine Cheng, Wang-Da Liu, Chiao-Wen Huang, Wen-Chun Liu, Chien-Ching Hung
National Taiwan University Hospital, Taipei, Taiwan

Background: We previously showed in a clinical trial that PWH who had achieved virologic and immunologic responses with ART and were randomized to receive three double-dose (40-µg) HBV vaccine (Engerix) achieved a significantly higher seroresponse rate (anti-HBs antibody titer ≥10 mIU/ml) and high-titer (≥100 mIU/ml) seroresponse rate than PWH who received three standard-dose (20-µg) HBV vaccine. Factors associated with loss of seroprotection during follow-up were investigated.

Methods: PWH who were born after 1986 and achieved seroresponses at Wk 28 following HBV revaccination in the trial were included in the follow-up. Loss of seroprotection was defined as testing negative for HBsAg and anti-HBc with anti-HBs titer <10 mIU/ml. The clinical characteristics at revaccination and during follow-up were analyzed, including age, sex, smoking status, CD4 count, plasma HIV RNA, and pre-vaccination anti-HBs titer.

Results: 228 seroresponders (mean age, 28.5 years; median CD4, 599 cells/mm³) after HBV revaccination were included, 112 receiving standard-dose and 116 double-dose HBV vaccine. The baseline characteristics were balanced between the two groups. The median follow-up time was 2.48 and 2.04 years for PWH receiving standard-dose and PWH receiving double-dose HBV vaccine, respectively. During the follow-up, more PWH in the standard-dose group lost seroprotection than those in the double-dose group (22.3% vs 11.2%, p=0.024). Loss of high-titer seroresponse was more common for the standard-dose group than the double-dose group (44.3% vs 19.8%, p=0.001). Factors associated with loss of seroprotection during follow-up were older age (aHR, 1.14 [95% CI, 1.03-1.27], p=0.010), lower pre-revaccination anti-HBs titer (<2.5 mIU/ml, aHR 13.23 [95% CI, 3.15-55.47], p<0.001), and HBV vaccine dose (double-dose, aHR 0.42 [95% CI, 0.21-0.90], p=0.024). People failing to achieve high-titer responses (10-99 mIU/ml) at Wk 28 were more likely to lose seroprotection during follow-up (HR 27.9 [13.7-57.0], p<0.001) with a median time to lose anti-HBs titer being 1.26 years when compared with people achieved high-titer responses (≥100 mIU/ml) at Wk 28.

Conclusion: Three double-dose HBV revaccination leads to more sustained seroprotection than standard-dose revaccination in PWH. For PWH who fail to achieve high-titer seroresponse after revaccination, annual follow-up of anti-HBs titers is recommended to detect the loss of seroprotection for HBV revaccination to be administered timely.

730 Chronic Hepatitis B Increases Mortality Risk in COVID-19 While Vaccination Is Protective
George A. Yendewa1, Temitope Olasehinde1, Frank Mulindwa1, Amir M. Mohareb1, Jeffrey Jacobson2
1Case Western Reserve University, Cleveland, OH, USA, 2University Hospitals Cleveland Medical Center, Cleveland, OH, USA, "United Health Services Wilson Medical Center, Johnson City, NY, USA, "Harvard Medical School, Boston, MA, USA

Background: Chronic hepatitis B virus (HBV) infection is considered a risk factor for severe SARS-CoV-2 infection (COVID-19); however, there is conflicting evidence regarding its impact on COVID-19 outcomes in dually infected individuals. Furthermore, while COVID-19 vaccination has been associated with a lower risk of death and adverse outcomes in the general population, its effect on COVID-19 outcomes in individuals with chronic HBV infection remains unexplored.

Methods: We used the TriNetX database to compare adult patients with confirmed SARS-CoV-2 infection and chronic HBV (COVID-HBV) and without chronic HBV (COVID-wo-HBV) who sought care across 77 healthcare systems in the United States from January 2020 to August 2023. We included people with HBV diagnosis codes and laboratory testing. We assessed the risk of inpatient hospitalization, intensive care unit admission, mechanical ventilation, early (30-day) and late (90-day) mortality. We further assessed the impact
of COVID-19 vaccination on outcomes in subgroup analysis of COVID-HBV. We addressed potential confounders using 1:1 propensity score matching by demographics, key comorbidities, and COVID-19 vaccination. For outcomes of interest, we calculated odds ratios (OR) and 95% confidence intervals (CI), with statistical significance set at p < 0.05.

**Results:** Of 3,360,173 individuals with confirmed SARS-CoV-2, about 0.2% (7,163) were COVID-HBV, of which 13.9% (996) were vaccinated. People with COVID-HBV had higher odds of 90-day mortality (OR 1.21, 95% CI 1.02-1.44; p = 0.02) and ICU admission (OR 1.39, 95% CI 1.17-1.66; p < 0.001) compared with COVID-wo-HBV; however, there was no significant difference between groups in 30-day mortality, hospitalization, and mechanical ventilation rates. In subgroup analysis of COVID-HBV, those who received COVID-19 vaccination had lower odds of death at 30 days (OR 0.38, 95% CI 0.22-0.66; p < 0.001) and 90 days (OR 0.46, 95% CI 0.31-0.70; p < 0.001). Vaccination was not associated with decreased odds of hospitalization, ICU admission, and mechanical ventilation rates.

**Conclusion:** In the largest study to date, chronic HBV infection conferred higher odds of death and adverse outcomes in COVID-19. Notably, COVID-19 vaccination was associated with a significant reduction in the odds of death and the need for ICU admission, suggesting that vaccination could be an effective strategy for mitigating the impact of COVID-19 in individuals with chronic HBV infection.

**Table 1. Clinical outcomes in COVID-19 vs COVID-wo-HBV and effect of COVID-19 vaccination on outcomes**

<table>
<thead>
<tr>
<th>Impact of HBV on COVID-19</th>
<th>Effect of COVID-19 Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcomes</td>
<td>COVID-19 (n=343)</td>
</tr>
<tr>
<td>Death (30-day)</td>
<td>179 (5.1%)</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>234 (6.3%)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>240 (6.7%)</td>
</tr>
<tr>
<td>Liver transplantation</td>
<td>30 (0.9%)</td>
</tr>
<tr>
<td>Liver transplantation</td>
<td>26 (0.7%)</td>
</tr>
<tr>
<td>Graft rejection</td>
<td>2 (0.1%)</td>
</tr>
</tbody>
</table>

**IL-21 Ameliorates Liver Inflammation by Enhancing MDSC Activity in Chronic HBV Infection**

**Xiaoyi Li, Zhipeng Liu, Guofu Ye, Shihong Zhong, Yongyin Li, Shen Li, Yongzheng Hu, Kelsey L. Overman, Xue Li, Hongzhou Lu, Chuanzhong Gu, Shuqin Gu, Guofu Ye, Yongyin Li**

**Background:** In patients with chronic HBV infection, the immune response is inadequate for HBV clearance but can cause a persistent inflammatory reaction. Alleviating hepatitis may be possible by inhibiting the function of inflammatory cells during hepatitis activity. The potential for interleukin 21 (IL-21) to suppress the liver inflammation by regulating the suppressive activity of myeloid-derived suppressor cells (MDSCs) on T cell response is not well understood.

**Methods:** Eighty treatment-naive patients with chronic HBV infection were recruited and classified into the immune tolerant carrier (IT; n = 37), hepatitis B/HBeAg (HBV) carriers (IC; n = 27) groups according to the American Association for the Study of Liver Diseases guidelines. Another 33 healthy controls (HCs) were also enrolled in this study. We investigated the characteristics of MDSCs and the effect of IL-21 on the phenotype and function of MDSCs by flow cytometry. The quantity of granulocytic MDSCs (gMDSCs) in the human liver was analyzed by immunofluorescence assay. Wild-type C57BL/6 mice were used to establish a wild-type C57BL/6 mouse model.

**Results:** The frequency of circulating MDSCs in patients infected with chronic HBV was higher than that in the HC group. The circulating MDSCs frequency was negatively correlated with the serum levels of ALT and AST. The number of CD15 and CD66b double-positive cells indicating gMDSCs in CHB patients was higher than that in IT and IC patients. Exogenous IL-21 promoted MDSCs differentiation into M2-like macrophages while also increasing their expression of Arginase I in MDSCs than mice injected with blank plasmid.

**Conclusion:** Our study highlights the robust immune-suppressive activity of MDSCs, which can be enhanced by exogenous IL-21, and their potential role in protecting against liver inflammation in chronic HBV infection. These findings provide a novel perspective on the involvement of IL-21 in the negative regulation of intrahepatic inflammation and have implications for the development of immunotherapeutic strategies to optimize the available anti-inflammatory hepatica in chronic HBV infection.
733 Novel Biomarkers as Determinants of HBsAg Loss in Persons With HIV/HBV on Tenofovir
Lorin Begre1, Anders Boyd2, Marie-Laure Plissonnier, Barbara Testoni3, Charles Béguelin4, Franziska Suter-Riniker5, Caroline Scholtes1, Juergen R. Rockstroh6, Karine Lacombe1, Lars Peters7, Massimo Leverdo1, Andri Rauch8, Fabien Zoulim8, Gilles Wandel8
1University Hospital of Bern, Bern, Switzerland, 2Public Health Service Amsterdam, Amsterdam, Netherlands, 3Université Claude-Bernard Lyon 1, Lyon, France, 4University of Bern, Bern, Switzerland, 5Bonn University Hospital, Bonn, Germany, 6Assistance Publique–Hôpitaux de Paris, Paris, France, 7University of Copenhagen, Copenhagen, Denmark
Background: HBsAg loss is associated with improved clinical outcomes in persons with hepatitis B virus (HBV) infection. The association between novel biomarkers, including circulating HBV RNA and hepatitis B core-related antigen (HBeAg), and this outcome has not been studied in persons with HIV/HBV. We aimed to evaluate rates of HBsAg loss and associated risk factors in Euro-B, a multi-cohort collaboration including participants from the Swiss HIV Cohort Study and EuroSIDA.

Methods: We included participants with a positive HBsAg and ≥6 months of follow-up on tenofovir-containing antiretroviral therapy (ART), who had quantitative HBV RNA (qHBV) levels measured at tenofovir start. We evaluated rates of HBsAg loss, defined as a negative qualitative test or qHBsAg <0.05 IU/mL, after 2 years of tenofovir therapy and at the last follow-up visit. We assessed risk factors for HBsAg loss on tenofovir overall and in HBeAg-stratified analyses using multivariable logistic regression. Due to collinearity between HBeAg and HBV RNA, we evaluated risk factors in two separate multivariable logistic regression models.

Results: Of 304 participants included, 23.0% experienced HBsAg loss during a median follow-up time of 11 years (IQR 6-15). After two years, 37/265 (14.0%) participants had experienced HBsAg loss. At tenofovir start, median age was 41 years (IQR 36-46), 61/304 (20.1%) were female at birth, 152/304 (50.0%) were ART-naïve, median CD4 count was 325 cells/mm^3 (IQR 210-462), and 109/233 (46.8%) were HBeAg positive. At tenofovir start, 72/304 (23.7%) participants had a qHBsAg <1000 IU/mL, 79/304 (26.0%) had HBV DNA <20 IU/mL, 72/304 (23.7%) had HBeAg c <3.0 log IU/mL, 104/304 (44.6%) had HBV RNA <10 copies/mL. In both models, only qHBsAg <1000 IU/mL at baseline was associated with HBsAg loss (model with HBeAg: OR 12.6, 95% CI 5.4-29.5; model with HBV RNA: OR 9.6, 95% CI 4.3-21.4). In HBeAg-positive participants, HBV RNA (OR 0.5 for 1 log increase, 95% CI 0.3-0.8) was associated with HBsAg loss. In HBeAg-negative participants, only qHBsAg <1000 IU/mL (OR 10.8, 95% CI 3.1-36.9) was associated with this outcome.

Conclusion: We found high rates of HBsAg loss among persons with HIV/HBV, with 14% of events occurring during the first two years of treatment. Baseline qHBsAg levels were strongly associated with HBsAg loss in HBeAg-negative participants, whereas HBV RNA may be an independent predictor of HBsAg loss in HBeAg-positive persons with HIV/HBV.

734 Circulating HBV RNA Helps Characterize HBV Disease in Senegal
Lorin Begre1, Hubert Akotia2, Bruce Wembulua Shinga3, Melissa S. Pandi4, Ousseynou Ndiaye5, Judith Horstmann6, Pascal Bittler7, Christoph Niederhauser8, Martin Stolz9, Andri Rauch10, Ndeye Fatou Ngom11, Moussa Seydi12, Gilles Wandel13
1University Hospital of Bern, Bern, Switzerland, 2Cheikh Anta Diop University, Dakar, Senegal, 3Centre Hospitalier Universitaire de Famen, Dakar, Senegal, 4University of Bern, Bern, Switzerland, 5Interregional Blood Transfusion SRC, Bern, Switzerland
Background: Hepatitis B virus (HBV) infection affects >10% of the population in West Africa and is the most common cause of liver cirrhosis and hepatocellular carcinoma. Circulating HBV RNA may help improve the characterization of HBV disease and prognosis. We aimed to evaluate the associations between HBV RNA and conventional biomarkers of HBV replication in the Senegalese Hepatitis B Cohort study (SEN-B).

Methods: We included all treatment-naive participants without HIV infection from SEN-B. We measured HBV RNA levels using the cobas HBV RNA investigational assay (Roche Molecular Diagnostics, Pleasanton, CA) with a lower limit of quantification of 10 copies/mL. Participants were classified into three phases of HBV infection: Hepatitis e antigen (HBeAg)-positive (EP), HBeAg-negative chronic infection (ENCI) and HBeAg-negative chronic hepatitis (ENCH). ENCH was defined by HBV DNA >2000 IU/mL in combination with elevated alanine aminotransferase (ALT > 30 IU/mL for men, >19 IU/mL for women) and/or significant liver fibrosis (liver stiffness measurement >7.0 kPa). We compared participants with and without detectable HBV RNA using descriptive statistics and evaluated associations between HBV RNA, HBV DNA, and quantitative hepatitis B surface antigen (qHBsAg) using Spearman’s rank correlation coefficient among participants with detectable HBV RNA levels.

Results: Among 713 participants, 17 (2.4%) were in the EP phase, 615 (86.3%) in the ENCI phase and 81 (11.4%) in the ENCH phase. Median age was 31 years (interquartile range 25-38) and 335 (47.0%) were female. HBV RNA was undetectable in 3/17 (17.6%) of EP individuals, 338/615 (53.0%) of ENCI individuals and 19/81 (23.5%) of ENCH individuals. The 333/713 (47.9%) participants with detectable HBV RNA were more likely to have HBV DNA >20 IU/mL (95.8% vs. 80.0%) and to have significant fibrosis (13.9% vs. 7.5%) than participants with undetectable HBV RNA levels. We did not observe significant differences in age, sex, ALT levels and qHBsAg levels between the two groups. As depicted in the Figure, HBV RNA showed strong correlation with HBV DNA in EP, moderate correlation in ENCH and poor correlation in ENCI participants. HBV RNA and qHBsAg correlated moderately in EP, but not in ENCI and ENCH participants.

Conclusion: In our cohort of treatment-naive persons with HIV in Senegal, approximately 50% had detectable HBV RNA levels. HBV RNA correlated well with HBV DNA in the EP and ENCI groups, whereas qHBsAg levels correlated with HBV RNA only in EP individuals.

735 Efficacy and Safety of BLV 2 or 10 mg for 96 Weeks in CHD Including in 2 Patients With HIV/HBV/HDV
Heiner Wedemeyer1, Seo Aleman2, Maurizia Bruzzone3, David L. Wyles3, Viacheslav Morozov4, Vladimir Chulanov5, Ben Da6, Renee-Claude Mercier7, Grace M. Chee8, Mingyang Li9, Pietro Lampertico10
1Medizinische Hochschule Hannover, Hannover, Germany, 2Karolinska Institute, Stockholm, Sweden, 3University Hospital of Pisa, Pisa, Italy, 4Gilead Sciences, Inc, Foster City, CA, USA, 5Medical Company Hepatology, Samara, Russian Federation, 6Federal Budget Institute of Science, Moscow, Russian Federation, 7Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy
Background: Bulevirtide (BLV) is a first-in-class, entry inhibitor, approved in the EU for the treatment of chronic hepatitis D. Limited data exists on the safety and efficacy of BLV in HIV/HBV/HDV coinfected. The aim of this Week (W) 96 analysis from the Phase 3 MY301 (NCT03852719) study is to describe the safety and efficacy of BLV over 96 weeks including in patients with HIV/HBV/HDV.

Methods: 150 patients with CHD were randomized (1:1:1) into three arms: Arm A: no active anti-HDV treatment for 48 weeks, followed by BLV 10mg/d for 96 weeks (n=51), and Arms B or C: immediate treatment with BLV at 2 mg/d (n=49) or 10 mg/d (n=50), respectively, each for 144 weeks, with follow-up of 96 weeks after end of treatment. Efficacy was measured by combined (undetectable HDV RNA or ≥2 log IU/mL decline from BL and ALT normalization), virologic and biochemical response rates. Controlled HIV infection (defined as CD4 count >500/mL, HIV RNA <LLOD) was allowed.

Results: Overall, baseline characteristics were similar between arms: mean (SD) age 41.8 (8.4) years; 57% males; 83% White; 47% compensated cirrhosis; mean (SD) HDV RNA 5.05 (1.34) log10 IU/mL, ALT 110.9 (69.0) U/L, and 67% on concomitant nucleos(t)ide analogue. Two patients with HIV/HBV/HDV were enrolled in the study. At baseline, Patient 1 (Arm B) was age 39 years, HCV
Transaminase Elevations Among Patients With Occult HBV Infection on 2-Drug Antiretroviral Regimens
Luca Mezzadri, Baisa Monti, Alice Ranzani, Anna Cappelletti, Silvia Limonta, Alessandro Soria, Elisa Coletta, Ilaria C. Caramma, Nicola Squillace, Paolo Bonfanti, Giuseppe Lapadula
Fondazione IRCCS San Gerardo di Dentro, Monza, Italy
Background: Concerns have been raised about the possibility of hepatitis reactivation among individuals with occult HBV infection (OHBVI) who discontinue antiretroviral agents with anti-HBV activity. Whether OHBVI is associated with increased risk of transaminase elevation among those switching to two-drugs regimens (2DR), discontinuing lamivudine (3TC) and/or tenofovir (TIV), merits to be investigated.
Methods: People living with HIV (PLWH) who had switched to 2DR since 2018 were enrolled in this retrospective cohort, provided they had discontinued ≥1 anti-HBV drug and that their anti-HBV core (HBc) status was known. Rates of liver function test increase (LFTI) above the upper limit of the normal range were determined using Kaplan-Meier estimates and compared between those with and without OHBVI. Cox regression, adjusting for potential confounders, and random-effects linear regression with repeated measures were also used. All analyses were conducted separately according to the type of 2DR used (including 3TC or without any active HBV-agents).
Results: 162 patients switched to a 2DR containing 3TC while 105 to a regimen without 3TC and TIV. Their characteristics are shown in Fig 1. Among those on 3TC, incidence of LFTI was 13.3 and 16.8 per 100 person-years in those with and without OHBVI, respectively (IRR 0.78; 95% CI 0.35-1.75; p=0.42). Uni and multivariable Cox regression showed no significant association between OHBVI and LFTI (HR 0.8; 95% CI 0.4-1.8; p=0.55), after adjusting for possible confounders. No association between ALT levels and OHBVI was observed using a random-effects linear regression, adjusted for time and baseline ALT (coefficient -0.39, 95% CI -2.3-1.5). Among those with no anti-HBV agents in the regimen, LFTI rates were 13.9 and 13.9 per 100 patient-years in those with and without OHBVI, respectively (IRR 0.99; 95% CI 0.31-3; p=0.989). Using Cox regression, no significant association between OHBVI and LFTI was found (HR 1.33, p=0.568). Adjusted models for potential confounders yielded similar findings. Similarly, OHBVI was not significantly associated at linear regression analysis (coefficient -0.87, 95% CI -3.8-2.1). No clinical HBV reactivations nor acute infections were observed.
Conclusion: Presence of OHBVI infection was not significantly associated with transaminase elevation among PLWH treated with 2DR lacking anti-HBV agents. This real-life observation provides reassurance regarding the safety of transitioning to dual therapy in patients with reactive anti-HBC. The figure, table, or graphic for this abstract has been removed.

737 Hepatitis B Reactivation in PLWH With Anti-Core Antibody After Switch to an Anti-HBV Sparing Regimen
Giaia Morsica1, Riccardo Lolatto1, Sara Diotallevi1, Valentina Sveer, Costanza Bertoni1, Alessia Sinibelli, Hamid Hasson1, Sabrina Bagaglio1, Tommaso Clemente1, Arianna Forini1, Romina Salpini1, Caterina Uberti-Foppa1, Antonella Castagna1, Nicola Giansiti1, San Raffaele Scientific Institute, Milan, Italy, 2University of Rome Tor Vergata, Rome, Italy, 3Vita-Salute San Raffaele University, Milan, Italy, 4University of Florence, Florence, Italy
Background: Anticore antibody (antiHBC) in absence of hepatitis B surface antigen (HBsAg) is defined as an isolated antiHBc status that may be associated with HBV reactivation. We decided to investigate HBV reactivation in antiHBC-PLWH (with isolated anti-HBc or with anti-HBc and anti-HBsAg) after the switch from ART including drugs active on both viruses (tenofovir disoproxil fumarate, TDF and tenofovir alafenamide, TAF) to an antiHBV sparing regimen (antiHBVsr) because in PLWH the loss of HBsAg with or without anti-HBsAg seroconversion is probably consequent to ART treatment including drugs active on both viruses (HIV/HBV) rather than past HBV infection. Additionally, HBV reactivation has been described in immunocompromised antiHBc positive people.
Methods: HBV reactivation in antiHBC-PLWH was assessed by an alteration of alanine aminotransferase (ALT) above the upper limit of the normal range after the switch to antiHBVsr Dolutegravir/rilpivirine (DOL/RPV) or long acting therapy cabotegravir/rilpivirine (CAB/RPVLA). Forty-one individuals with antiHBc and at least 2 ALT values available after the switch to antiHBVsr: (DOL/RPV, number, N=15), CAB/RPVLA(N=26) were evaluated. Data were collected as part of routine clinical care and recorded into the database of the Division of Infectious Diseases of the San Raffaele Fate (CLSHV Cohort). Data freezing was 29 August 2023.
Results: Main characteristics of antiHBc-PLWH at antiHBVsr according to antiHBc status (antiHBcAg positive N=34 or negative, N=7) are described in Table 1. All variables analyzed were similarly distributed in the two groups. Overall, the median post-switch follow-up was 8.91 months (interquartile range, IQR 6.78 - 24.14). 8.96 months (IQR 6.78 - 24.14) in antiHBcAg positive PLWH and 6.97 months (IQR 5.56 - 43.22) in antiHBcAg negative PLWH, P=0.742. Interestingly, 1/7(14.3%) PLWH with isolated antiHBc showed an increase of ALT (about 20-fold the normal values) about 3 months after switch to CAB/RPVLA, with also appearance of HBV-DNA at high levels (4.8 x107 IU/mL) and HBsAg seroconversion. He was infected by HBV genotype A that was also identified in plasma sample obtained during HBV chronic infection phase, before any ART treatment.
Conclusion: The HBV reactivation is unlikely in antiHBC-PLWH with anti-HBcAg positivity after switch to anti-HBVsr, while close monitoring of ALT and possibly HBV-DNA in PLWH with antiHBc alone, after switch to anti-HBVsr is necessary.

738 Circulating HBsAg-Specific B-Cells Are Partially Rescued After Achieving Functional Cure
Shuqin Gu1, Li Tao2, Guang Ye1, Ling Guo1, Shihong Zhang1, Xianyi Li4, Nikolai Novikov1, Simon P. Fletcher1, Sarah Valencich1, M. Anthony Moody1, Yongjin Li1, Duke Human Vaccine Institute, Durham, NC, USA, 2Southern Medical University Nanfang Hospital, Guangzhou, China, 3Southern Medical University Nanfang Hospital, Guangzhou, Guangdong, China, 4Gilead Sciences, Inc, Foster City, CA, USA
Background: Hepatitis B surface antigen (HBsAg) loss defines a functional cure for chronic hepatitis B infection. Herein, we aimed to characterize circulating
HBsAg-specific B cells and identify B-cell epitopes directly associated with HBsAg loss. 

Methods: Seventy-eight treatment-naive patients with chronic HBV infection were classified into 4 groups according to the EASL guidelines (2017): hepatitis B e antigen (HBeAg) negative chronic infection (n=21), HBeAg-positive chronic infection (n=20), HBeAg-negative chronic hepatitis (n=17), and HBeAg-positive chronic hepatitis (n=20). Forty-six patients who achieved HBeAg loss (n=24) or seroconversion (n=22) following antiviral treatment and 16 hepatitis B vaccinees with normal alanine aminotransferase levels were enrolled. HBsAg-specific B cells-phenotypes and function were analyzed by flow cytometry. ELISPOT assay was used to measure the ability of B cells to secrete antibodies. HBsAg-specific B-cell epitopes associated with functional cure were mapped by ELISA.

Results: The classical resting memory B cell population (RM, CD21+/CD27+) in HBsAg-specific B cells was reduced while the proportion of atypical memory B cells (ATM, CD21-CD27-), a functionally exhausted subset, was expanded in chronically HBV-infected patients. An increased proportion of RM B cells and a decreased proportion of ATM B cells were observed in patients after achieving functional cure. There was no correlation between total HBsAg-specific B cells and virological parameters, but we found an inverse correlation between HBsAg levels and HBsAg-specific RM B-cell frequency. Moreover, HBsAg-specific B cells expressed lower levels of CD32 and higher levels of IL-6 upon stimulation in patients with HBsAg loss. Intriguingly, the frequency of HBsAg-secretory B cells was significantly increased after achieving functional cure. Sera from patients with HBsAg loss mainly reacted with peptides 560, 561, and 567, suggesting that they are dominant linear B-cell epitopes relevant for functional cure. Notably, an expansion of HBsAg-specific B cell was detected in S76-reactive subjects, accompanied by lower PD-1 expression.

Conclusion: HBsAg-specific B cells were partially restored in patients after achieving functional cure. Functional cure-related epitopes are promising targets of therapeutic vaccine development for chronic HBV infection to increase the functional cure rate.

Evaluation of the Hepatitis B Virus Reservoir With Fine Needle Aspiration and Serum Biomarkers

Barbara Testoni1, Armando Andres Roa Suarez2, Marie-Laure Plissomnier3, Caroline Scholtes3, Floriana Facchetti2, Manzita Heil1, François Villereit2, Yasmina Chouik1, Maximo Leverero2, Upkar Gill1, Patrick Kennedy4, Pietro Lampertico4, Fabien Zoulim5

1L’Université Claude Bernard Lyon, Lyon, France, 2Fondazione IRCCS Ca’ Granda Ospedale Maggior Pollicita, Milano, Italy, 3Icole Molecular Systems, Inc, Pleasanton, CA, USA, 4Barts Liver Centre, London, United Kingdom

Background: Novel treatment strategies aimed at hepatitis B virus (HBV) cure must eliminate or silence the covalently closed circular (ccc)DNA reservoir, which measurement is challenging as it requires invasive sampling methods. To address this unmet medical need, we evaluated the HBV reservoir by: i) analyzing viral markers in fine needle aspirates (FNAs) as a less invasive alternative to core liver biopsy (CLB), and ii) quantifying circulating HBV RNA (cir-B-RNA) as an indicator of cccDNA transcriptional activity.

Methods: We collected matched CLB/FNA/serum samples from chronic hepatitis B patients (n=9), as well as paired CLB/serum samples from untreated (n=92) and nucleos(t)ide analog (NUC)-treated (n=30) individuals. Intrahepatic viral parameters were quantified by qPCR and droplet digital (dd)PCR (LOD 3 x 10-6 copies/cell for cccDNA), cir-B-RNA was quantified with the automated cobas(R) HBV RNA assay that preferentially detects viral RNAs derived from cccDNA vs. integrated sequences (LOD 5 copies/mL). Serum levels of HBV DNA, hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcAg) were determined, as well as hepatitis B e antigen (HBeAg) status.

Results: cccDNA and 3.5-kb RNA were quantifiable in all but one CLB/FNA pair, showing the highest levels in untreated HBeAg+ patients. When comparing cccDNA and 3.5-kb RNA levels in CLB/FNA samples, no statistically significant differences were identified. The analysis of CLB/serum samples showed that all HBeAg+ chronic hepatitis (CH) patients had quantifiable cir-B-RNA, compared to only 57% of HBeAg-CH and 14% of HBeAg-chronic infection (CI) untreated patients and 47% of NUC-treated patients. cir-B-RNA undetectability was associated with lower intrahepatic cccDNA transcriptional activity and serum HBcAg in both untreated and treated patients. Combined undetectability of cir-B-RNA and HBcAg in HBeAg- patients identified a subgroup with the lowest levels of transcriptionally active cccDNA.

Conclusion: In the frame of HBV cure programs, we provide a proof of concept that the less invasive FNA can be used to assess intrahepatic cccDNA using ddPCR assay. Moreover, we show the performance and relevance of quantifying cir-B-RNA as an indicator of cccDNA transcriptional activity in both untreated and NUC-treated patients. These results support the use of both approaches in clinical trials to evaluate the HBV reservoir during the development of new antivirals and immunomodulatory agents.

Results:

739 Monthslong HBV Suppression by TFV Double Ester Prodrugs

Srijanee Das, Weimin Wang, Samiksha Raut, Murali Ganesan, Grace A. Bybee, Nam Thai Hoang Le, Howard E. Gendelman, Natalia A. Osna, Larisa Poluektova, Benson Edagwa

University of Nebraska Medical Center, Omaha, NE, USA

Background: Tenofovir (TFV) prodrugs [TFV disoproxil-/TFV alafenamide-fumarates (TDF/TAF)] are recommended daily oral treatments for chronic hepatitis B (HBV) infection. Given the growing popularity of long-acting (LA) therapies for treatment of chronic viral infections, an ever increasing number of potent medicines will come available. One, developed in our own laboratories, is a lipophilic ProTide of TFV called M1TFV that exhibited sustained suppression of HBV DNA in Tg05 mice without recorded efficacy compared to limited anti-HBV activity for TAF ProTide or arylated NNATFV diester prodrug (Figure 1).

Conclusion: NM5TFV and NM6TFV diester prodrug formulations produced sustained monthslong suppression of HBV DNA in Tg05 mice without recorded adverse events.

740 Evaluation of the Hepatitis B Virus Reservoir With Fine Needle Aspiration and Serum Biomarkers

Background:

Novel treatment strategies aimed at hepatitis B virus (HBV) cure must eliminate or silence the covalently closed circular (ccc)DNA reservoir, which measurement is challenging as it requires invasive sampling methods. To address this unmet medical need, we evaluated the HBV reservoir by: i) analyzing viral markers in fine needle aspirates (FNAs) as a less invasive alternative to core liver biopsy (CLB), and ii) quantifying circulating HBV RNA (cir-B-RNA) as an indicator of cccDNA transcriptional activity.

Methods:

We collected matched CLB/FNA/serum samples from chronic hepatitis B patients (n=9), as well as paired CLB/serum samples from untreated (n=92) and nucleos(t)ide analog (NUC)-treated (n=30) individuals. Intrahepatic viral parameters were quantified by qPCR and droplet digital (dd)PCR (LOD 3 x 10-6 copies/cell for cccDNA), cir-B-RNA was quantified with the automated cobas(R) HBV RNA assay that preferentially detects viral RNAs derived from cccDNA vs. integrated sequences (LOD 5 copies/mL). Serum levels of HBV DNA, hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcAg) were determined, as well as hepatitis B e antigen (HBeAg) status.

Results:

cccDNA and 3.5-kb RNA were quantifiable in all but one CLB/FNA pair, showing the highest levels in untreated HBeAg+ patients. When comparing cccDNA and 3.5-kb RNA levels in CLB/FNA samples, no statistically significant differences were identified. The analysis of CLB/serum samples showed that all HBeAg+ chronic hepatitis (CH) patients had quantifiable cir-B-RNA, compared to only 57% of HBeAg-CH and 14% of HBeAg-chronic infection (CI) untreated patients and 47% of NUC-treated patients. cir-B-RNA undetectability was associated with lower intrahepatic cccDNA transcriptional activity and serum HBcAg in both untreated and treated patients. Combined undetectability of cir-B-RNA and HBcAg in HBeAg- patients identified a subgroup with the lowest levels of transcriptionally active cccDNA.

Conclusion:

In the frame of HBV cure programs, we provide a proof of concept that the less invasive FNA can be used to assess intrahepatic cccDNA using ddPCR assay. Moreover, we show the performance and relevance of quantifying cir-B-RNA as an indicator of cccDNA transcriptional activity in both untreated and NUC-treated patients. These results support the use of both approaches in clinical trials to evaluate the HBV reservoir during the development of new antivirals and immunomodulatory agents.
741 Intrahepatic cccDNA and Circulating HBV Markers in HBeAg-Negative Chronic Infection in Senegal
Adriá Ramirez Mena, Aleksi Suslov, Hubert Akotie, Pascal Bittel, Andreas Limacher, Juditine Tietz, Christoph Niederhauser, Bruce Wembulua Shinga, Melissa S. Pandi, Ndeye Fatou Ngom, Stefan Wieland, Markus Heim, Moussa Seydi, Gilles Wandeler
University Hospital Basel, Basel, Switzerland, Centre Hospitalier Universitaire de Fann, Dakar, Senegal, University of Bern, Bern, Switzerland

Background: A better understanding of the relationship between the intrahepatic hepatitis B virus (HBV) activity and peripheral biomarkers of HBV infection is urgently needed in order to improve treatment monitoring and outcomes in high prevalence settings. We aimed to determine the relationship between intrahepatic HBV cccDNA and plasma HBV DNA, quantitative HBsAg (qHBsAg) and total HBV RNA levels in HBeAg-negative individuals from the SEN-B cohort in Senegal.

Methods: We collected paired core liver biopsies and serum/plasma samples from untreated HBeAg-negative participants enrolled in SEN-B. We measured qHBsAg and HBV DNA on site using e411 cobas® and cobas® TaqMan® (Roche Diagnostic Systems). HBV RNA levels were measured from cryopreserved plasma samples using cobas® 8800 investigational assay (Roche Molecular Systems) with a lower limit of quantification (LLQ) of 10 copies/mL. cccDNA was extracted from snap-frozen liver samples using modified protocol, followed by ExoV nuclease treatment, and quantified by an HBV specific digital droplet PCR. The individual correlation between levels of intrahepatic cccDNA and i) HBV DNA, ii) qHBsAg and iii) HBV RNA was assessed using Spearman correlation coefficients.

Results: Fifty SEN-B participants were included with a median age of 31 years (interquartile range 26-37) and 16/50 (32%) were female. One individual (3%) had ALT >40 IU and 13/50 (26%) had liver fibrosis defined as a Metavir stage ≥F2. HBV DNA >2,000 IU/mL was found in 19/50 (38%) participants and qHBsAg >1,000 IU/mL in 28/50 (56%). HBV RNA was detected in 26/50 (52%) participants, of whom 12/50 (24%) had >10 copies/mL. Median cccDNA (copies/cell) was similar between HBV RNA-negative and positive individuals (p=0.74). We observed no significant correlation between the intrahepatic cccDNA and plasma HBV DNA levels (r=0.21, p=0.15), as well as between the intrahepatic cccDNA and serum qHBsAg levels (r=0.02, p=0.87). There was a moderate positive correlation between cccDNA and total plasma HBV RNA among persons with detectable levels (r=0.52, p=0.006).

Conclusion: HBV RNA was the only circulating marker which correlated with intrahepatic cccDNA in our well-characterized group of persons with HBeAg-negative HBV infection in Senegal. Further research is needed to better understand the underlying mechanisms and implications of this correlation. The figure, table, or graphic for this abstract has been removed.

742 Race/Ethnicity and Risk of NAFLD and Clinically Significant Fibrosis in Persons Living With HIV
Timsay A. Woerter, Mark Sulkowski, Yuchun Xin, Laura Wilson, Eduardo Vilar-Gomez, Samer Gavrieleh, Kathleen Corey, Jennifer Price, Susanna Nagpie, Sonya Heath, Richard Sterling, James Tonascia, Rohit Loomis, Naga Chalasani, Jordan E. Lake
The Johns Hopkins University School of Medicine, Baltimore, MD, USA, The Johns Hopkins University, Baltimore, MD, USA, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, Indiana University, Indianapolis, IN, USA, Massachusetts General Hospital, Boston, MA, USA, University of California San Francisco, San Francisco, CA, USA, Duke University School of Medicine, Durham, NC, USA, University of Alabama at Birmingham, Birmingham, AL, USA, University of Virginia Commonwealth University, Richmond, VA, USA, University of California San Diego, San Diego, CA, USA, University of Texas at Houston, Houston, TX, USA

Background: Racial and ethnic differences in non-alcoholic fatty liver disease (NAFLD) prevalence are well described in the general population, with Hispanics having the highest and African Americans having the lowest NAFLD prevalence. Whether similar disparities exist in persons with HIV (PWH) with and without HIV-1 RNA viral suppression is currently unknown.

Methods: This cross-sectional analysis included participants ≥18 years of age prospectively enrolled in two U.S. multicenter studies from 2018-2023 who had: 1) a documented history of HIV on antiretroviral therapy with HIV-1 RNA <200 copies/mL; 2) a self-reported race/ethnicity group listed as non-Hispanic White (NHW), non-Hispanic Black (NHB), or Hispanic. NAFLD was defined by a controlled attenuation parameter (CAP) score ≥263 dB/m in the absence of excessive alcohol consumption and other causes of liver disease. CSF was defined as a liver stiffness measurement (LSM) ≥8 kPa.

Results: Of 457 individuals enrolled, 365 (80.5%) with ≥2 valid VCTE measurements were included. Median follow-up time was 30 months (IQR 23-37), 105 participants (23.1%) were female and 261 (70.9%) Caucasian. At time of first VCTE, their median age was 52 years (interquartile range [IQR] 43–59), median CD4+ count was 728 cells/μl (IQR 547–939), and 193 (53.3%) had a BMI≥25 kg/m². Median ART duration was 11 (IQR 6-19) years and 344 (93.5%) had a HIV viral load <50 copies/mL. At first VCTE, 190 participants (51.6%) had liver steatosis, of whom 6 (3.2%) showed steatohepatitis with significant fibrosis. We investigated risk factors for de novo steatosis using multivariable logistic regression.

Conclusion: Of 457 individuals enrolled, 365 (80.5%) with ≥2 valid VCTE measurements were included. Median follow-up time was 30 months (IQR 23-37), 105 participants (23.1%) were female and 261 (70.9%) Caucasian. At time of first VCTE, their median age was 52 years (interquartile range [IQR] 43–59), median CD4+ count was 728 cells/μl (IQR 547–939), and 193 (53.3%) had a BMI≥25 kg/m². Median ART duration was 11 (IQR 6-19) years and 344 (93.5%) had a HIV viral load <50 copies/mL. At first VCTE, 190 participants (51.6%) had liver steatosis, of whom 6 (3.2%) showed steatohepatitis with significant fibrosis. We investigated risk factors for de novo steatosis using multivariable logistic regression.
Among 190 participants with steatosis at first VCTE, 6 (3.1%) developed steatohepatitis with significant fibrosis during follow-up. **Conclusion:** During three years of follow-up, one in four PWH developed de novo steatosis, with obesity and dyslipidemia being the most important predictors. The progression of liver steatosis to steatohepatitis with significant fibrosis was rare.

**744 A Gut Microbiome Signature for HIV and Non-Alcoholic Fatty Liver Disease**

Javier Martínez-San1, Alba Talavera-Rodríguez2, Jorge Díaz-Arévalo1, Marta Rosas Cancín-Suárez1, Juan Miguel Rodríguez-Gómez1, Claudio Sanz1, María Luisa Montes1, Rosa Martín-Mateos1, Diego Burgos-Santamaría1, Santiago Moreno1, Sergio Serrano-Villar1, Matilde Sánchez-Conde1

1Hospital Ramón y Cajal, Madrid, Spain. 2Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain.

**Background:** Non-alcoholic fatty liver disease (NAFLD) has emerged as an increasingly recognized problem among people living with HIV (PLWH). The gut-liver axis is considered to be strongly implicated in the pathogenesis of NAFLD. We aimed to characterize the gut microbiota composition in PLWH and NAFLD and compare it with that of two control groups: PLWH without NAFLD and individuals with NAFLD without HIV infection.

**Methods:** We collected clinical data and stool samples from participants. Bacterial 16S rRNA genes were amplified, sequenced, and clustered into operational taxonomic units. Alpha diversity was studied by the Shannon and Simpson indexes. To study how different the gut microbiota composition is between the different groups, beta diversity estimation was evaluated by principal coordinate analysis (PCoA) using Bray-Curtis dissimilarity. To further analyze differences in microbiome composition, we performed a linear discriminant analysis (LDA) effect size (LEfSe). We selected the genus with a LDA score >4.

**Results:** We included 30 HIV+NAFLD+, 30 HIV+NAFLD- and 20 HIV-NAFLD+ participants. Major butyrate producers, including Faecalibacterium, Ruminococcus, and Lachnospira, dominated the microbiota in all three groups. Shannon’s and Simpson’s diversity metrics were higher among NAFLD+ individuals (Kruskal-Wallis p = 0.047). Beta diversity analysis showed distinct clustering in NAFLD+, with NAFLD+ participants overlapping regardless of HIV status (ADONIS significance <0.001) (Figure 1A). NAFLD was associated with increased homogeneity among individuals, in contrast to that observed in the HIV+NAFLD- group, in which the dispersion was higher (Permanova test, p value <0.001; ANOSIM, p value <0.001). NAFLD but not HIV determined a different microbiota structure (HIV+NAFLD+ vs. HIV+NAFLD-, q-value = 0.002; HIV−NAFLD+ vs. HIV+NAFLD+, q-value = 0.930; and HIV−NAFLD+ vs. HIV+NAFLD+, q-value < 0.001). The most abundant genera in NAFLD+ were Prevotella, Bacteroides, Dialister, Acidaminococcus, Allroprevotella, and Catenibacterium. In contrast, the most enriched genera in NAFLD+ were Ruminococcus, Streptococcus, Holdemana, Blautia, and Lactobacillus

**A)**

**Conclusion:** We found a microbiome signature linked to NAFLD, which had a greater influence on the overall structure of the gut microbiota than HIV status alone. We suggest that part of the alterations in the microbiota described as associated with HIV could be confused by the presence of NAFLD, which is more prevalent in people with HIV.

**745 Liver Steatosis Is Not Associated With Endothelial Function or Hormone Use in Transgender Women**

Emilia M. Jalli1, Rodrigo C. Moreira1, Hugo Perazzo1, Marcelo Cunha1, Ronald Moreira1, Layla Monteiro1, Monica D. Pedrosa1, Biancka Fernandes1, Valdilea Veloso1, Beatriz Grinsztejn1, Sandra W. Cardoso2

1Osvaldo Cruz Foundation - Fiocruz, Rio de Janeiro, Brazil. 2University of Texas at Houston, Houston, TX, USA.

**Background:** Transgender women (TGW) present disproportionate chronic diseases burden. Nevertheless, very little is known about inflammation-related chronic diseases among TGW in low and middle-income countries (LMIC), especially in the context of HIV infection and gender-affirming hormone therapy (GAHT). We aimed to assess the association of liver steatosis with subclinical atherosclerosis measured by brachial artery flow-mediated dilatation (FMD) and GAHT.

**Methods:** Cross-sectional study among transgender women aged 18+ years of the Transcending cohort, Rio de Janeiro, Brazil, between October 2019-May 2023. Participants answered a structured questionnaire, collected blood samples, and performed transient liver elastography by Fibroscan and FMD. Valid elastography results were considered if: had at least 10 valid measurements, the percentage of valid measurements was >60%, and Controlled Attenuation Parameter (CAP) interquartile range (IQR)/CAP <30%. Results with CAP ≥248 dB/m were classified as liver steatosis. Percentage of FMD ([peak diameter-baseline diameter]/baseline diameter) was used for analysis. Bivariate analysis compared TGW with and without liver steatosis.

**Results:** Among 157 TGW, 31 (83.4%) had valid liver assessments. Among these, median age was 39.0 years (IQR:31.0-44.0), 80(61.0%) were living with HIV, 65(51.0%) were currently on GAHT, and 36(27.5%) had liver steatosis.

**Conclusion:** Traditional factors and HIV-positive status were associated with liver steatosis among Brazilian TGW. Changes in arterial diameter and GAHT exposure were not associated with liver steatosis. Our findings reinforce the need of including traditional factors of liver steatosis for TGW’s clinical assessment. This study was partially funded by Viiv Healthcare UK Ltd. The figure, table, or graphics for this abstract has been removed.

**746 Increased levels of FGF21 and GDF15 Are Associated With Severity of NAFLD in People With HIV**

Paula Debroy1, Francis Pike2, Samer Gaurieh3, Kathleen Corey4, Ashok Balasubramanyam5, Kate Allstock1, Nicholas Funderburg1, Jordan E. Lake6

1University of Texas at Houston, Houston, TX, USA. 2Indiana University, Bloomington, IN, USA. 3Massachusetts General Hospital, Boston, MA, USA. 4Bayler College of Medicine, Houston, TX, USA. 5The Ohio State University, Columbus, OH, USA.

**Background:** Non-alcoholic fatty liver disease (NAFLD) poses a significant health burden in people with HIV (PWH). Fibroblast growth factor 21 (FGF21) is an important regulator of hepatic lipid and glucose metabolism. Higher levels
have been associated with hepatic steatosis and liver fibrosis in the general population. Growth differentiation factor 15 (GDF15) is upregulated in chronic inflammatory diseases and associated with cardiovascular dysfunction in PWH. We aimed to describe the trends of FGF21 and GDF15 concentrations in PWH and NAFLD.

Methods: Consent of PWH and no other known cause of liver disease underwent vibration-controlled transient elastography for controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) quantification at three US centers. NAFLD was defined as CAP ≥263 dB/m; advanced fibrosis as LSM > 12 kPa. Fasting serum FGF21 and GDF15 were measured by ELISA. Relationships between biomarkers and NAFLD were analyzed using a Censored Tobit Model.

Results: Participants (n=177) had median age 52 years and were 20% cisgender women, 81% overweight/obese, 90% virally suppressed on antiretroviral therapy. Participants with NAFLD (50%) had significantly higher mean (SD) levels of FGF21 [337 (377) vs 226 (305) pg/ml, p = 0.002] and GDF15 [859 (371) vs 744 (366) pg/ml, p = 0.02] than participants without NAFLD. FGF21 levels increased with BMI (p=0.04). Higher FGF21 and GDF15 levels correlated modestly with higher CAP (FGF21 r=0.30, p<0.001; GDF15 r=0.21, p=0.01) and LSM scores (FGF21 r=0.25, p<0.001; GDF15 r=0.27, p=0.01). FGF21 concentrations were 40% higher (mean Log Difference 95% confidence interval = 0.34 (0.06, 0.62), p=0.02) and GDF15 17% higher (0.16 [0.01, 0.32], p=0.04) in persons with vs. without NAFLD. Participants with the highest FGF21 levels (quartile 4) had higher NAFLD prevalence (77% vs 39%, p=0.01), higher mean CAP (301 vs 247 dB/m, p=0.001) and LSM (6.2 ± 4.5 kPa, p=0.004) values, and longer mean duration of HIV (10 vs 17 years, p<0.001) compared to persons in quartile 1. Similar trends were seen with GDF15 level quartiles.

Conclusion: PWH and NAFLD had higher levels of FGF21 and GDF15 than those without NAFLD, with higher levels correlating with greater liver steatosis and fibrosis. FGF21 and GDF15 may have a role in identifying PWH at risk of metabolic liver disease. Further research is needed to elucidate the role of these circulating factors in PWH and NAFLD and their diagnostic and prognostic value.

747 Plasma Proteomics Signature of People Living With HIV. With and Without Obesity

Louise E. van Eekeren, Nadira Vadanq, Yasukii Matsuzaki, Adriana Navas, Eline M. Meeurder, Marc Blaauw, Willem A. Vos, Albert L. Groenendijk, Gert Weijers, Jan van Lunzen, Mihail Neta, Andre J. van der Veer, Quirijn de Mast, Eric T. Tiwa, Leo Joosten

Background: Non-alcoholic fatty liver disease (NAFLD) is a leading cause of liver-related morbidity in people living with HIV (PHLV). Obesity is an important risk factor but NAFLD also occurs in lean PHLV, with possible different underlying pathophysiology. Proteomics facilitate biomarker discovery and may help identify biological mechanisms.

Methods: We analyzed data from 1050 PHLV with transient elastography measurements from the 200NHV cohort, a Dutch multi-center study amongst virally suppressed PHLV (NCT03994835). Proteomics was available (Olink® Explores, 2367 proteins) from 1036 individuals (98.7%). Differentially expressed proteins (DEP) were compared between PHLV with and without liver steatosis (controlled attenuation parameter ≥ 248 dB/m) and with and without liver fibrosis (liver stiffness measurement ≥ 7.0 kPa) using a limma model. We stratified by BMI group (lean [BMI < 25 kg/m²] or < 23 kg/m² in PHLV from Asian descent) vs. overweight/obese PHLV (other BMI). Functional pathways from the GO biological processes, KEGG and Reactome libraries enriched with DEP were identified using Metascape. DEP were correlated to HIV-specific characteristics and antiretroviral therapy (ART).

Results: Plasma proteome of PHLV with steatosis or fibrosis were altered from those without steatosis or fibrosis, respectively. DEP in PHLV with steatosis were enriched in metabolic pathways, whereas DEP in PHLV with fibrosis were enriched in interleukin-10 (IL-10) signaling, cell-adhesion, and amino acid metabolism. More DEP (n = 27) were identified in lean PHLV with versus those without steatosis compared to overweight/obese PHLV (n = 15), and only four DEP were shared between lean and overweight/obese PHLV. i.e. IGFBP2, GHR, AFM, and the novel protein IGSF9. IGSF9 was also DE in PHLV with fibrosis. Especially in lean PHLV, multiple DEP were associated with HIV-characteristics including HIV duration and CD4 nadir and current ART (NNRTI and INSTI).

Conclusion: PHLV with steatosis and fibrosis have an altered plasma proteome including upregulation of proteins involved in metabolism, cell-adhesion and IL-10 signaling. Steatosis signatures differed between lean and overweight/obese PHLV. Furthermore, our findings indicate involvement of HIV-specific factors in the pathogenesis of lean NAFLD, and we discovered the potentially novel NAFLD biomarker IGSF9.

748 WT1 Upregulation by Lytic Induction of Kaposi Sarcoma Herpesvirus

Aya A. Talab, Yun Yeong Jang, Roby Gumenick, Ariene Ouedraogo, Shaun Hinds, Leslie Monorch, Ethel Cesarman

1 Weill Cornell Medicine, New York, NY, USA, 2 Cornell University, Ithaca, NY, USA

Background: Kaposi Sarcoma herpesvirus (KSHV or HHV8) is the etiologic agent of Kaposi sarcoma and is associated with Multicentric Castleman disease and Primary effusion lymphoma. KSHV demonstrates two different phases of infection in the host: latent and lytic which are both essential to KSHV pathogenesis. The viral protein regulator or transcription activator (RTA) is required for the latent to lytic switch. In previous work, we have demonstrated that de novo KSHV latent infection and in particular vFLIP upregulates Wilms’ Tumor 1 (WT1) expression along with its oncogenic isoforms known to have various functions contributing to tumorigenesis. The expression and functions of WT1 have not been explored during lytic KSHV infection.

Methods: Cell culture models of latent and lytic KSHV infection were utilized, including ISK-LAC-BAC16 cells latently infected with KSHV as well as HuART-1 and HUEV4 E4 endothelial cells that were infected with KSHV. ISK-LAC-BAC16 cells, which express RTA under a doxycycline-inducible promoter, were treated with sodium butyrate and doxycycline 2ug/ml for lytic reactivation. HuART-1 and HUEV4 were treated with sodium butyrate to induce the lytic program. WT1 siRNA was transfected in the ISK-LAC-BAC16 and HUEV4 E4 model systems using Lipofectamine RNAiMAX.

Results: Here we have found that upon induction of lytic reactivation of KSHV-infected ISK-LAC-BAC16 cells, WT1 is upregulated significantly and in particular oncogenic isoforms. Significant WT1 upregulation is also noted upon treatment with sodium butyrate of de novo KSHV infected HuART-1 and HUEV4 E4 endothelial cells. Using the ISK-LAC-BAC16 and HUEV4 E4, we also demonstrate that upon WT1 knockdown during lytic reactivation, there is noted further increase in vFLIP, LANA, and K8.1 viral gene expression.

Conclusion: These findings suggest that both latent and lytic KSHV infection upregulate WT1 expression. Furthermore, given the findings that WT1 knockdown during lytic reactivation leads to marked upregulation of viral gene expression, our data suggests that WT1 may play a complex role in regulating these two phases of infection that we are exploring further in ongoing studies.

749 Saliva Kaposi Sarcoma Herpesvirus Levels as a Diagnostic Marker of Visceral Kaposi Sarcoma

Matthew Witterholt, Tishya Carey, Kathryn Lurain, Ralph Mangusan, Anaïda Widell, Irene Ekvede, Vickie Marshall, Nazzarena Labo, Kyle Moore, Wendell Miley, Romin Roshan, Elena M. Cornejo Castro, Denise Whitby, Robert Yarchoan, Ramya Ramsawami

National Cancer Institute, Bethesda, MD, USA

Background: Kaposi sarcoma (KS), an HIV-associated malignancy, is caused by Kaposi sarcoma herpesvirus (KSHV). KS commonly affects the skin but can also lead to symptomatic visceral manifestations in the pulmonary and gastrointestinal (GI) tracts; it requires invasive procedures such as bronchoscopy or endoscopy for diagnosis. We assessed the outcomes of patients (pts) with visceral KS and evaluated the saliva KSHV viral load (VL) as a diagnostic marker.

Methods: We conducted a retrospective study of pts with a history of KS from the HIV and AIDS Malignancy Branch who underwent bronchoscopy and/
or endoscopy/colonnoscopy for symptoms concerning pulmonary and GI KS between 2005-2023. Diagnosis of pulmonary KS was based on imaging and visualization of large airway lesions consistent with KS on bronchoscopy. GI KS was confirmed with a biopsy following diagnostic procedures. KSHV VL DNA was quantified in saliva and peripheral blood using primers for the KSHV K6 gene, while cell number was calculated using human endogenous retinovirus 3 primers. Receiver operator curves were created to observe sensitivity and specificity of KSHV saliva VL to diagnose visceral KS among pts with visceral KS symptoms. Overall Survival (OS) was estimated using Kaplan–Meier analysis from date of KS diagnosis to death or last follow-up.

**Results:** Sixty pts (57 with HIV diagnosis, 77% on antiretroviral therapy, median (med) HIV VL of 87 copies/mL and CD4 T cell count of 125 cells/μL underwent procedures to identify visceral KS. Twenty-six had either pulmonary or GI KS (pulmonary= 8, GI= 18), 15 had both GI and pulmonary KS, and 19 had skin KS without visceral KS. Of those with any visceral KS, 51.2% had other concurrent KSHV-associated disorders. The med KSHV VL in the saliva among those with visceral KS was 1 (IQR: 1, 67566) copy/10^6 cells and was 767 (IQR: 629, 8455) copies/10^6 cells in the peripheral blood. A saliva KSHV VL level of >190 copies/10^6 cells had a sensitivity of 46%, specificity of 76%, and a positive likelihood ratio of 2 for predicting the presence of visceral disease among symptomatic pts. Median OS in pts with skin KS only was not reached, was 9.2 years in pts with GI or pulmonary KS, and 2.6 years in pts with both GI and pulmonary KS (P=0.009, Figure 1).

**Conclusion:** Despite well controlled HIV among pts with KS, the presence of visceral KS impacts overall survival. Saliva KSHV VL may be a useful tool to aid in diagnosis of visceral KS in limited-resource settings.

**Figure 1:** OS Kaplan–Meier curve comparing different KS patient groups.

### Baseline KSHV T-Cell Responses Are Associated With Pre-Treatment Clinical Presentation in KS


*University of Pittsburgh, Pittsburgh, PA, USA, 1Harvard TH Chan School of Public Health, Boston, MA, USA, The Johns Hopkins University, Baltimore, MD, USA, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 2CH Corporation, Silver Spring, MD, USA, 3University of Zimbabwe, Harare, Zimbabwe, 4University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 5Memorial Sloan Kettering Cancer Centre, New York, NY, USA*

**Background:** T cell immunity is important in controlling KS herpesvirus (KSHV) disease progression. We evaluated T cell responses to KSHV and associations with pre-treatment clinical characteristics and outcomes in 2 ACTG/AMC studies (AS5263/AMC066, AS5264/AMC067) of KS treatment in resource-limited settings. **Methods:** T cell ELISPOT responses (log_{10} spot-forming units (SFU)/10^6 cells) to 2 latency (LANA, K12) and 2 lytic (gB and K8.1) KSHV and to HIV Gag peptides were measured from 49 advanced KS (AS5263/AMC066) and 94 mild/moderate KS (AS5264/AMC067) participants with samples. In AS5264/AMC067, we also measured T cell PD-1 expression by flow cytometry. Wilcoxon rank-sum and Fisher’s exact tests were used.

**Results:** At entry, median age was 34; mostly male and black African. Median CD4 count was 250 and 190 cells/mm^3, and median plasma HIV-1 RNA was 3.5 and 5.1 log_{10} copies/mL in advanced and mild/moderate KS, respectively. Advanced KS participants with oral KS had lower baseline responses to K12 (median: 0.76 vs 2.30 log_{10} SFU; p=0.042), K8.1 (0 vs 2.32 SFU; p=0.038), and LANA (2.15 vs 3.02 SFU; p=0.039) than those without. In mild/moderate KS, a higher proportion of participants without oral KS had positive response to LANA (65% vs. 19% p=0.013). Advanced KS participants with Karnofsky scores (KPS) <90 had higher gB responses than those with KPS ≥90 (median: 2.55 vs 0 SFU; p=0.046). Similarly, in mild/moderate KS, a higher proportion of participants with KPS <90 had detectable responses to gB (63% vs. 21%; p=0.040). Despite strong responses to Gag, this response was not associated with oral KS or KPS. There was a trend for mild/moderate participants with screening T0 KS stage to respond to gB compared to those with T1 stage (54% vs 17%; p=0.056). Mild/moderate participants with oral KS had a trend for higher %PD1+CD8+ T cells (median 18.45% vs 14.40%; p=0.074) than those without. At 12 weeks, a higher proportion of mild/moderate participants without early KS disease progression had a drop in %PD1+CD4+ (27% vs 21% with early disease progression; p=0.002) and %PD1+CD8+ T cells (89% vs 57%; p=0.042). KSHV T cell responses were not associated with clinical outcomes in either study.

**Conclusion:** In advanced and mild/moderate KS, pre-treatment KSHV-specific T cell responses were associated with several pre-treatment clinical characteristics. In mild/moderate KS, decreased T cell exhaustion was associated with less early KS disease progression. This supports T cell immunity’s importance in controlling KS.

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**Description of Clonal Hematopoiesis in a Hospital-Based Cohort of People Living With HIV**

Manasa Bhatta, Myvishni Esi Selvan, Daniel I. Nathan, Nikolaos Spyrou, Zeynep Gumus, Bridget Marcellino, Keith Sigel

*Johns School of Medicine at Mt Sinai, New York, NY, USA*

**Background:** In the antiretroviral therapy era, the population of people living with HIV (PLWH) is aging, with increased mortality from chronic diseases and non-AIDS defining cancers. Similarly, clonal hematopoiesis (CH) has been associated with both malignancy and cardiovascular disease in the non-HIV population. Clonal hematopoiesis has been described as infrequent in patients under 50 and becomes more prevalent in the aging population. Recent studies have demonstrated that PLWH have a higher rate of CH than people without HIV. We aimed to describe CH in a cohort of PLWH as the initial phase of an ongoing analysis to determine the prognostic implications of CH in PLWH.

**Methods:** We used phenotype and whole exome sequencing data from patients recruited to the Mount Sinai Biome Biobank. We identified 487 patients in the cohort who met criteria for HIV. CH was called by excluding any patient with a myeloid malignancy and by filtering for predefined myeloid CH mutations with variant allele frequency (VAF) ≥ 2%.

**Results:** Out of 487 total patients with HIV, we identified 29 patients with HIV and 30 CH mutations. The median age of patients without CH was 49, while the median age of patients with CH was 57. Out of 29 of patients with CH were below the age of 50. In logistic regression analysis, age was significantly associated with presence of CH in the HIV population (p=0.006) while ancestry, smoking, and sex were not. The most common CH mutations in the cohort were DNMT3A (8/30) and TET2 (4/30). None of the patients had mutations in ASXL1. Median VAF was 8.4% (IQR 6.6 – 11.7%).

**Conclusion:** Our findings show a seemingly young age distribution of patients with CH in our cohort of PLWH, though we have not yet compared our cohort to a non-HIV population. Distinct from previously reported findings that ASXL1 was the most commonly mutated gene, we did not identify any mutations in ASXL1 in our cohort. Instead, other common CH genes such as DNMT3A and TET2 represented the majority of mutations. Interestingly, smoking, a commonly implicated risk factor for CH, was not significantly associated with presence of CH in our cohort. Multivariable analyses are ongoing with plans to investigate
CH in patients with HIV as a predictor of the risk of cardiovascular disease, malignancy, and death.

752 Inflammatory Signatures Predict the Risk of Severe Non-AIDS Events, Especially Non-AIDS Cancers

Javier Martínez-Sanz, Claudio Díaz-García, Elena Morenés, Laura Martín-Pedraza, Laura Luna García, Juan Carlos López Bernardo de Quiroga, Jose I. Bernardino, Marta Montero, Enrique Bernal, Helena Alibendi Iglesias, Santiago Moreno, Sergio Serrano-Villar, for the Cohort of the Spanish HIV/AIDS Research Network (CoRIS) Hospital Ramón y Cajal, Madrid, Spain, Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain, Hospital Universitario Gregorio Marañón, Madrid, Spain, Hospital La Paz Institute for Health Research, Madrid, Spain, Hospital Universitario La Fe, Valencia, Spain, Hospital Universitario Reina Sofia, Murcia, Spain, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain.

Background: While elevated levels of inflammatory biomarkers in people with HIV (PWH) have consistently been linked to a higher risk of severe non-AIDS adverse events (SNAEs), their clinical significance and the relevant inflammatory pathways are not well-established.

Methods: In this nested case-control study within the Spanish AIDS Research Cohort (CoRIS), from a pool of 18,573 PWH we selected 89 cases who experienced SNAEs after two years of ART (including cardiovascular events, non-AIDS-related cancers, or non-accidental deaths) and had available plasma at month 24 (±6) of suppressive ART. Cases were matched with 89 controls using propensity-score matching. Covariates included age, sex, factor for HIV transmission, AIDS history, geographical origin, year 2 CD4/CD8 ratio, baseline HIV RNA, ART regimen, total cholesterol and HDL cholesterol. We measured the expression of 368 inflammatory proteins in plasma using Olink Proteomics’ Proximity Extension Assay and analyzed their functions in Metacapse. We used Welch 2-sample t-tests at a confidence level of 0.95 for every protein for a given outcome and corrected for multiple testing by the Benjamini-Hochberg method.

Results: We studied 178 PWH, median age 45 years, 25% women, 49% smokers, median CD4 nadir 206 cells/μL, median CD4 counts at year 2 469 cells/μL. We found significant changes in the expression of inflammatory proteins before month 24 subsequently predicting SNAEs risk. In cases, 25 proteins were upregulated, and 8 were downregulated. The greatest size effect was found for CLIP2, SKAP2, DAPP1, MANF, with approximately two-fold increased expression upregulated, and 8 were downregulated. The greatest size effect was found for CLIP2, SKAP2, DAPP1, MANF, with approximately two-fold increased expression

Conclusion: Infection, inflammation signaling pathways, immune response activation, and cancer-related pathways, including NF-κB, PD-1, and B-cells.

753 Starting ART Early After HIV Acquistion Reduces Long-Term Non-AIDS-Defining Malignancy Risk

Iris A. van der Walp, Ferdinand Wirt, Peter Reiss, Marc Van der Valk

‘Amsterdam Institute for Global Health and Development, Amsterdam, Netherlands, ‘Stichting HIV Monitoring Amsterdam, Netherlands, ‘University of Amsterdam, Amsterdam, Netherlands.

Background: Non-AIDS Defining Malignancies (NADM) have become a prominent cause of death in people with HIV (PWH). Evidence shows that starting antiretroviral treatment (ART) at a higher CD4 count is associated with reduced NADM risk. We aimed to investigate whether starting ART early after acquiring HIV reduces NADM risk even further.

Methods: We included PWH 18 years or older from the Dutch National ATHENA cohort without a known NADM diagnosis starting ART between 1/1/2000–31/12/2022. NADM and infection-related NADM were analyzed separately. Individuals who started ART ≤365 days of a last known negative HIV test or with a documented primary infection (Fiebig stages 1-5) were categorized as “Early-ART”, and all others as “Late-ART” starters. Hazard Ratios (HR) for NADM were estimated by unadjusted and adjusted Cox regression. Models were adjusted for traditional (age, sex at birth, calendar time, HIV transmission category, smoking (time-updated), and region of origin) and HIV related (time-updated CD4 count lagged by 3 months, CD4/8 ratio and time spent with HIV RNA >1000 copies/ml) factors.

Results: Early-ART compared to Late-ART starters were younger (median age 34.7 vs. 39.4 years), more often male (94.3 vs. 80.0%), with a CD4 count >500 cells/mm³ at ART start (42.6 vs. 18.8%), less often current or former smokers (39.7 vs. 44.9%), and had shorter median follow-up (68 vs. 115 months). In the Early-ART starters (n=2,036) 28 NADM occurred during 12,454 PYFU (IR 2.2/1000 PYFU) versus 1,160 NADM during 220,237 PYFU (IR 5.3/1000 PYFU) in Late-ART starters (n=22,183). Unadjusted, Early-ART start was associated with a significantly reduced NADM hazard (HR 0.48 [95% Confidence Interval (CI) 0.33–0.69]), which was only moderately attenuated after adjustment (multivariate HR 0.63 [95% CI 0.43–0.92]). When only considering infection-related NADM, 8 events occurred in Early-ART starters (n=2,037) during 12,531 PYFU (0.6/1000 PYFU) versus 378 during 223,390 PYFU (IR 1.7/1000 PYFU) in Late-ART starters (n=22,252). Early-ART start was associated with a significantly lower infection-related NADM hazard (HR 0.38 [95% CI 0.19–0.79]) in unadjusted analysis. In our multivariable model results were similar to the all-NADM analysis, but lacked statistical significance (HR 0.64 [95% CI 0.31–1.29]).

Conclusion: Starting ART within a year of acquiring HIV or during primary infection reduces the risk of NADM compared to starting ART later after infection. Larger studies should assess the impact on individual NADM types.

754 Prevalence of Cancer Screening in People With HIV Utilizing a Symphony Health Data Linkage in Texas

Jennifer K. McGee-Avila, Eric Engels, Cameron B. Haas, Qianlai Luo, Marie-Joséphe Horner, Meredith Shiels

‘National Cancer Institute, Rockville, MD, USA, ‘National Cancer Institute, Bethesda, MD, USA

Background: Cancer risk is elevated in people with HIV (PWH) compared to those without HIV. Screening for cancer can identify cancer at earlier stages where curative therapy can be most efficacious, or, in the case of cervical and colorectal cancers, identify precancerous lesions that can be treated, preventing progression to malignant cancer. For PWH, cancer screening is important given higher incidence of late-stage disease at diagnosis, disparities in receipt of cancer treatment, and elevated risk of cancer related mortality.

Methods: Data from the HIV/AIDS Cancer Match (HACM) Study, a population-based HIV and cancer registry linkage was linked to Symphony Health, an...
aggregator of health data including claims from medical, laboratory, hospital, and physician records, in Texas during 2008-2015. Among PWH, we estimated the prevalence of anal, breast, cervical, colorectal, and prostate screening using International Classification of Diseases, Ninth and Tenth Revision (ICD-9/10) diagnosis codes, Current Procedural Terminology (CPT), Healthcare Common Procedure Coding System (HCPCS) codes, and revenue codes. For each screening type we limited the cohort to those who were screening eligible based on age criteria as of 2008, and then estimated ever screening during 2008-15.

Results: We included a total of 51,670 people with HIV with linked claims data. Approximately 77.6% of PWH were male, and racial/ethnic breakdown was 39.8% Non-Hispanic (NH) Black, 36.6% NH White, 21.9% Hispanic, and 1.8% Other/Unknown. For age distribution, 16.8% was aged 18-29, 24.6% aged 30-39, 35.7% aged 40-49, 18.0% aged 50-59, 4.3% aged 60-69, and 0.6% aged 70-80. For women with HIV aged 30 and older, 38.5% received cervical cancer screening and similarly, 40.0% had ever received screening for breast cancer, between the ages of 40-74 during the observation period. Approximately 31.5% of men with HIV, between the ages of 55-69 received prostate cancer screening. For PWH between the ages of 45-75, approximately 26.7% received screening for colorectal cancer. Lastly, while official guidelines do not exist for the general population, among PWH between the ages of 35-70, only 0.96% received screening for anal cancer.

Conclusion: Findings suggest utilization of screening modalities for anal, breast, cervical, colorectal, and prostate cancer are underutilized among PWH in Texas. More work is needed to determine barriers to receiving screening.

755 Racial Disparities in Cancer Incidence Among Men Who Have Sex With Men With HIV in the US, 2001-2019

Benton G. Meldrum, Meredith Shieff, Jennifer K. McGee-Avila, Tyler Adamsom, Qianlai Luo, Tabassum Insaf, Ruth Pfeiffer, Eric Engels, Cameron B. Haas

National Cancer Institute, Rockville, MD, USA, ‘Maryland Department of Health and Mental Hygiene, Baltimore, MD, USA,’ New York State Department of Health, Albany, NY, USA

Background: Men who have sex with men (MSM) are disproportionately affected by HIV, representing 68% of new diagnoses in 2019. Racial inequities in access to HIV screening, diagnosis, and treatment result in lower prevalence of viral suppression for non-white MSM. We hypothesize that higher risk of progression to AIDS is likely to increase cancer risk among non-white MSM with HIV (MSMWH).

Methods: We investigated racial disparities in cancer risk among MSMWH from 2001-2019 using the HIV/AIDS Cancer Match Study data. We examined 9 cancer types: prostate, lung, liver, anal, non-Hodgkin lymphoma, oropharyngeal, Kaposi sarcoma, colon, and Hodgkin lymphoma. We calculated age-adjusted incidence rates and standardized incidence ratios (SIR) by dividing observed cases by expected cases based on rates in the US population within region, age group, and year. We stratified these calculations by AIDS status.

Results: Overall, excess risk of each cancer type varied significantly by race/ethnicity. We examined 9 cancers (see figure), but these results will only discuss anal cancer because rates are particularly elevated among MSMWH. Non-Hispanic (NH) white MSM had an overall age-adjusted incidence rate of 87.4 per 100,000 person-years (95% CI = 84.8, 89.9), significantly higher than NH Black MSM (68.8, 95%CI =66.1,71.5), and Hispanic/Latino MSM (50.3, 95%CI=47.8, 52.7). When stratified by AIDS status, those with a prior AIDS diagnosis were 3 times higher for NH white and NH Black MSM, but 4.5 times higher for Hispanic/Latino MSM, compared to those without. For SiR, NH white MSMWH had 38.4 (95% CI = 36.3, 40.6) times the risk of anal cancer compared to all NH white men in the US population. NH Black and Hispanic/Latino MSM had 24.1 (95% CI = 22.3, 26.0) and 30.1 (95% CI = 28.0, 33.8) times the risk, respectively. These SIRs were greater among all races/ethnicities for MSM with a prior AIDS diagnosis compared to MSM without.

Conclusion: Incidence rates and relative risks compared to the general population differed significantly by race/ethnicity. When stratified by AIDS diagnosis, SIRs and SIR ratios were significantly different between races/ethnicities. Notably, risk of anal cancer was only 2.5 times higher for non-Hispanic white MSM diagnosed with AIDS compared to HIV, but 3.5 times higher for Hispanic/Latino MSM. Hispanic/Latino MSM often had the highest SIRs suggesting a significantly higher excess risk among Hispanic/Latinos or potential under-ascertainment of cancers in the general population.

756 Prevalent and Incident Cancers in a Cohort of People With HIV in Latin America

Valeria I. Fink, Carina Cesar, Shengain Tu, Bryan E. Shepherd, Claudia P. Cortes, Guillerme Calvet, Juan Sierra-Madero, Eduardo Gotuzzo, Diana Varela, Jessica L. Castilho

for the Caribbean, Central and South America Network for HIV Epidemiology (CCASAnet)

1Fundación Huesped, Buenos Aires, Argentina, 2Vanderbilt University, Nashville, TN, USA, 3University of Chile, Santiago, Chile, 4Instituto Nacional de Infectología Evandro Chagas, Rio de Janeiro, Brazil, 5Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, 6Universidad Peruana Cayetano Heredia, Lima, Peru, 7Instituto Hondureño de Seguridad Social, Tegucigalpa, Honduras

Background: Cancer is a leading cause of morbidity and mortality among people with HIV (PWH). Earlier HIV diagnosis and timely access to antiretroviral treatment (ART) have led to longer life expectations among PWH, resulting in an increase in aging-related comorbidities, including non-AIDS-defining cancers (NADC). While the epidemiology of NADC in PWH has been described in high resource settings, less is known in low- and middle-income settings. We evaluated the prevalance and incidence of NADC and AIDS-defining cancers (ADC) within the Caribbean, Central and South America network for HIV epidemiology (CCASAnet) and factors associated with these malignancies.

Methods: PWH ≥18 years old enrolled at CCASAnet sites in Argentina, Brazil, Chile, Honduras, Mexico and Peru from 2000-2020 were included. Prevalent (diagnosed any time before or <90 days after clinic entry) and incident (diagnosed ≥90 days after clinic entry) NADC and ADC were collected. Clinical and demographic factors associated with prevalent and incident ADC and NADC were evaluated using multivariable logistic regression and multi-state Cox proportional hazards models, respectively. Models included age, sex, HIV acquisition risk, site, year of cohort entry, and time-updated ART, CD4 cell count, and history of other respective cancer.

Results: In total, 2776 WPH were included. At clinic entry, median age was 33 years (IQR: 27.4-41), 78% were male, 37% had previous ART, and median CD4 cell count was 257 cells/ ml (IQR: 96-457). Prevalent ADC was present in 795 PWH, 101 had NADC and 7 had both ADC and NADC. Risk of prevalent ADC and NADC varied by site. Older PWH, those with lower CD4 count (<250 cells/ml), and HIV acquisition risk factors other than heterosexual contact were more likely to present with prevalent ADC and NADC (<p<0.05 for all). Those with male sex, earlier year of entry and prior ART were also more likely to present with ADC (p<0.05 for all). During a median of 3.9 (IQR: 1.0-9.4) years of follow up, there were 300 incident ADC and 264 NADC. Risk factors for incident ADC and NADC are shown in Figure 1.

Conclusion: Prevalent and incident ADC were more frequent than NADC among PWH in CCASAnet. Older age and lower CD4 were associated with prevalent and incident NADC and prevalent ADC. Recent years of cohort entry and higher CD4 count were associated with decreased risk of prevalent and incident ADC. These findings continue to underscore the importance of early HIV diagnosis and treatment.
757 Increased Cancer Risk With Low CD4 Counts Persists Despite Over 2 Years of Virological Suppression

Jennifer F. Hoy, for the RESPOND and D:A:D Study Groups
Alfred Hospital, Melbourne, Australia

Background: Antiretroviral therapy (ART) leads to a reduction in AIDS-related events and lower CD4 counts are associated with some non-AIDS events. The impact of long term virological suppression on non-AIDS related cancers (NADC) is unclear. We determined whether the most recent CD4 count was an independent predictor of incident cancer risk in people with HIV who had virologic suppression (VS) for at least 2 years.

Methods: Individuals from the D:A:D and RESPOND cohort collaborations who achieved 2 years of VS on ART were included. Follow-up was from baseline (date of VS for ≥2 years) until the earliest of a first cancer event, confirmed virological failure (>200 copies/mL) or cessation of ART for ≥2 months, final follow-up, or administrative censoring date (D:A:D: 2/1/2016; RESPOND: 12/31/2021).

Results: Overall, 51,622 people with VS were included (median [IQR] baseline age 44 years [37, 51], CD4 count 536 cells/µL [376, 729], nadir CD4 count 238 cells/µL [112, 386], 72% male, 36% current smokers). There were 2152 incident cancers during a total of 321,126 person-years of follow-up (PYFU), median 6 years [2.9, 9.5]). This included 276 ADC (0.86 [0.76, 0.97]/1000 PYFU), and 1876 NADC (5.84 [5.58, 6.11]). There were 721 infection-related (2.24 [2.08, 2.41]), 927 smoking-related (2.89 [2.7, 3.08]), and 491 BMI-related (1.53 [1.4, 1.67]) cancers, which were not mutually exclusive. After adjustment, there was a significant reduction in the adjusted IR ratio (aIRR) by higher time-updated CD4 count (<350, 350-499, 500-749, >750 cells/µL) stratified by pre-ART nadir CD4 count and adjusted for confounders determined a priori.

Conclusion: Despite being virologically suppressed on ART for >2 years, individuals with poorer immune recovery (CD4 <500 cells/µL) continue to experience a significantly higher incidence of all cancer groups. This underscores the importance of earliest possible diagnosis of HIV and prompt initiation of ART to ensure optimal sustained risk reduction of both ADC and NADC.

758 Incidence of Diffuse Large B-Cell Lymphoma in Relation to the HIV Epidemic in South Africa 2011-2021

Carole Metekou1, Mazvena Muchengeti1, Yann Ruffieux1, Patricia Kellett1, Matthias Egger1, Eliane Rohner1, Tracey Wiggill2
1University of Bern, Bern, Switzerland, 2National Health Laboratory Service, Johannesburg, South Africa, 3Stellenbosch University, Stellenbosch, South Africa

Background: Diffuse large B cell lymphoma (DLBCL) is HIV-associated and the most common type non-Hodgkin lymphoma worldwide. We examined the impact of the HIV epidemic and antiretroviral therapy (ART) roll-out on incident DLBCL in South Africa by comparing characteristics and temporal trends of incident DLBCL between the Black (high HIV prevalence) and the White (low HIV prevalence) populations.

Methods: We identified DLBCL diagnosed in South Africa from 2011-2021 in the pathology-based National Cancer Registry using International Classification of Disease Oncology, 3rd Edition morphology codes. Using direct standardization, we computed age-specific incidence rates and estimated yearly age-standardized incidence rates (ASIR). We used joinpoint regression to estimate annual percentage changes (APC) in the ASIR of DLBCL.

Results: In South Africa, 13,560 DLBCL were diagnosed from 2011-2021; 55% of them (n=7410) were among men. The median age at DLBCL diagnosis was 47 years (IQR=37-59). Two-thirds of incident DLBCL occurred in Black (65% [n=8790] and 22% in White individuals [n=3006]). The incidence of DLBCL was highest among middle-aged adults in the Black population and older White people (Figure A). The overall ASIR of DLBCL per 100,000 persons was 5.8 in the White and 1.8 in the Black population. The ASIR of DLBCL was higher in the White population across all calendar years (Figure B). In the Black population, we noted an annual increase of 5.9% (95%CI 0.02 to 37.1) from 2011-2017 with a declining trend thereafter (APC -4.2%; 95%CI -22.8 to 3.7). Similarly, in the White population, the ASIR of DLBCL showed a yearly increase of 5.6% (95%CI 4.8 to 7.9) from 2011-2019 with a declining trend thereafter (APC -3.4%; 95%CI -8.1 to 2.4).

Conclusion: Whereas incident DLBCL in the White population mostly occurs among elderly individuals, the high DLBCL incidence rates among middle-aged Black individuals in South Africa indicate that HIV primarily drives incident DLBCL in this population. However, despite the introduction of ART in 2004, the DLBCL rates continued to increase in the Black population for more than a decade and only decreased one year after introducing the universal-test-and-treat policy in 2016. This suggests that wide coverage and timely initiation of ART are needed to reduce the DLBCL incidence in the Black population in South
The heterogeneity of risk for invasive anal cancer (IAC) among individuals living with HIV (PWH) underscores the importance of identifying clinical predictors to inform a shared decision-making framework for screening. We investigated predictors of IAC and described outcomes among those diagnosed with IAC.

Methods: The study clinic assembled a longitudinal HIV infection cohort of anal cancer screening outcomes. Screening procedures included anal cytology, digital anorectal examination, high-resolution anoscopy (HRA), and access to ablative treatments. We computed the adjusted probabilities of developing IAC over 5 and 10 years since the initial screening anal cytology. We describe the clinical outcomes of those diagnosed with IAC. Cox proportional models with inverse probability weighting were fit to account for differential screening in the cohort and to construct a nomogram for predicting IAC risk.

Results: Between 2007 to 2020, 8139 PWH received care at UCSF Owen Clinic, of whom 4105 (49.8%) underwent at least 1 anal cytology test and were followed for a median of 5.5 years (up to 13 years). Among them, 32 individuals developed IAC (age range at diagnosis 29-76 years). Those diagnosed with IAC following their first anal cytology were more likely to be younger and exhibit anal high-grade squamous intraepithelial lesion (aHSIL) on initial or subsequent follow-up cytology. Additionally, they showed lower median and nadir CD4 cell counts and underwent anal ablative treatment less frequently. Analyses did not reveal differences in IAC development based on gender identity, race, ethnicity, HIV risk factor, or tobacco use. The highest risk of IAC was associated with PWH having nadir CD4 cell count less than 200 with a hazard ratio (HR) of 3.73 (95%CI 1.61-8.54) and cytology aHSIL (HR 3.75 1.47-9.47). PWH with a combined nadir CD4 cell count less than 200 and cytology aHSIL had a 5- and 10-year probability of IAC of 2.9% and 3.7%, respectively (Figure). Of 32 PWH diagnosed with IAC, 7 died due to cancer progression. All who died due to IAC had clinical outcomes of those diagnosed with IAC. Cox proportional models with inverse probability weighting were fit to account for differential screening in the cohort and to construct a nomogram for predicting IAC risk.

Conclusion: PWH with nadir CD4 cell count below 200 and cytology aHSIL have the highest risk of anal cancer within 5 years of follow-up. All patients who died had anal cancer clinical stages IIIA or higher at diagnosis, highlighting the importance of early diagnosis through HRA cancer surveillance.

760 Evaluation of the Performance of Different High-Resolution Anoscopy Triage Strategies in MSM LWH

Eugenia Nelson Cavallari1, Federica Alessi1, Chiara Eberspacher1, Marco Ridolfi1, Illyas El Abboubi2, Alessandra Latini1, Angelina Pernazz2, Daniela Bosco2, Domenico Mascagni2, Claudio Maria Mastroianni1, Gabriella D’Ettorre2

1Azienda Ospedaliero-Universitaria Policlinico Umberto I, Rome, Italy, 2University of Rome, Rome, Italy.

Background: Treatment of anal high grade squamous intraepithelial lesions (HSIL) have been proved to be effective in reducing the incidence of squamous cell carcinoma of the anus (SCCA) in men who have sex with men (MSM) living with HIV (LWH). High resolution anoscopy (HRA) is the gold standard for detection of anal HSIL. Access to HRA is still not widely available due to the small number of trained providers. Definition of triage pathways to better allocate HRA resources is crucial to implement SCCA screening programs for PLW.

Methods: To evaluate sensitivity (SE) and specificity (SP) of different HRA triage strategies, we retrospectively analyzed data from 180 MSM LWH that underwent SCCA screening with HRA directed biopsies and for whom anal cytology and anal HPV DNA collected on the same day of HRA were available. In the analysis, only HRA that led to the identification of HSIL were classified as positive. As hypothetical threshold for referral to HRA we tested: 1) cytology of atypical squamous cells of undetermined significance (ASC-US) or worse; 2) cytology of low grade squamous intraepithelial lesion (LSIL) or worse; 3) cytology of atypical squamous cells cannot exclude HSIL (ASC-H) or worse; 4) any high risk HPV genotype (HR-HPV); 5) HPV16; 6) ASC-US or worse + HR-HPV; 7) LSIL or worse + HR-HPV; 8) ASC-US or worse + HPV16.

Results: When considering cytology criteria as tirage to HRA: an ASC-US threshold would have triggered 125 HRA (SE 70.9%; SP 32.4%); an LSIL threshold would have triggered 107 HRA (SE 62.1%; SP 43.3%); an ASC-H threshold would have led to 10 HRA (SE 11.4; SP 99%). When considering anal HPV infection as tirage for HRA: a threshold of any HR-HPV would have triggered 105 HRA (SE 84.8%; SP 62.7%), while a threshold of anal infection with HPV16 would have led to 57 HRA (SE 50.6%; SP 83.3%). When considering a composite triage: a threshold of ASC-US or worse + HR-HPV would have led to 81 HRA (SE 64.6%; SP 70.6%); a threshold of LSIL or worse + HR-HPV would have triggered 65 HRA (SE 54.4%; SP 78.4%); a threshold of ASC-US or worse + HPV16 would have triggered 43 HRA (SE 38%; SP 87.3%).

Conclusion: Triage to HRA with the identification of anal HR-HPV infection offered the best balance between sensitivity and specificity. The association of anal cytology (ASCUS or worse, or LSIL or worse) and HR-HPV, which present lower sensibility and higher specificity, seem feasible given the periodic nature of such screening. Official approval of assays for the diagnosis of anal HR-HPV is needed.

761 Anal Self-Sampling Is Suitable for Anal Cancer Screening Among Men Who Have Sex With Men in Togo

Valentine M. Ferré1, Arnold Sadi2, Romane Guilbaud3, Myriem Zaidi2, Mawussé K. Attriti4, Moumouou Saloué5, Laurent Abramowitz6, Mélanie Bertiné7, Amnivi P. Amenyah-Ehlan1, Éphrem Mensah8, Claver Anoumou Dagorn9, Jade Ghosn10, Diane Descamps10, Didier Koumavi Ekouevé11, Charlotte Charpentier12

1Université Paris Ouest, Nanterre, France, 2Centre Africain de Recherche en Épidémiologie et en Santé Publique, Lomé, Togo, 3Assistance Publique–Hôpitaux de Paris, Paris, France, 4VIEO Espoir Vie Togo, Lomé, Togo, 5Université de Lomé, Lomé, Togo

Background: Anal cancer screening guidelines exist only locally or nationally for some at-risk population like MSM living with HIV. In Sub-Saharan Africa, there is no access to anal cytology analysis and proctologic consultations. There is a need to implement HPV detection strategy to screen most at-risk populations. The aim of this study was to evaluate anal self-sampling (ASS) for HPV detection compared to anal swab carried out by the practitioner (ASP).

Methods: The ANRS 12400 DepIST-H cohort included 200 MSM in Togo, half living with HIV, prospectively followed up with yearly anal sampling and proctologic exam. During the month-12 visit, ASS was proposed to MSM before clinical consultation. A flyier explaining the procedure accompanied the FlocSwab for ASS, which was discharged into eNAT. The practitioner conducted afterwards anal exam, anal sampling with a cytobrush discharged in ThinPrep. All samples were analyzed by the Virology lab of Bichat Hospital (Paris, France) with AnyplexII for detection of 14 high-risk HPV (HR-HPV). HPV16 viral load was quantified with in-house qPCR.

Results: A total of 188 MSM were included, median age of 23 years, 99% of participants found the ASS procedure was easy to carry out and 60% of
there would prefer ASS to ASP at next visit while 19% had no preference. ASS was suitable for HPV detection since only 5% samples were uninterpretable compared to 7% for ASP (p = 0.77). Overall, at least one HR-HPV was detected in 83% (n=148/178) and 77% (n=135/176) of ASP and ASS, respectively, and 28% and 26% were positive for HPV16. ASP and ASS showed substantial agreement (89.7%) for HP-HPV detection with Kappa's coefficient of 0.66). The agreement for HPV16 was 90.3% (Kappa's coefficient = 0.75). HPV16 median viral loads were higher in ASS than than (7652 c/mL vs 575 c/mL respectively, p = 0.009). Regarding the 16 samples with discordant result for HPV16 detection, HPV16 viral load was low (160 c/mL for ASS and 155 c/mL for ASP in median).

Conclusion: To our knowledge, this is the first time ASS and ASP are compared for HPV detection performance at the same time. The concordance of the two sampling methods, the acceptability of ASS and the facility to implement self-sampling are in favor of using ASS for HPV detection in anal cancer screening programs. The HPV detection implementation worldwide for cervical cancer screening following the WHO's 2020 guideline will enable anal cancer screening implementation in LMIC. The figure, table, or graphic for this abstract has been removed.

762 Long-Term ART Is Not Associated With Reduced Anogenital Cancer Risk: A Cohort Study
Maanas Mendu1, Taolu Ntloedibe2, Memory Bvuchora-Ngingo1, Sebathu Chiyapo1, Kutlo Manyake1, Isaac Nkole1, Rebecca Luckett2, Tendani Gaolathe2, Joseph M. Makhema3, Peter Vuyisile2, Shahin Lockman2, Scott Dryden-Peterson1
1Harvard University, Cambridge, MA, USA, 2Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 3Life Gaborone Private Hospital, Gaborone, Botswana, *Beth Israel Deaconess Medical Center, Boston, MA, USA, 4University of Botswana, Gaborone, Botswana

Background: People with HIV (PWH) are at increased risk of anogenital cancer. Malignancies of the cervix, vulva, anus, and penis have become leading causes of morbidity and death for PWH globally. HIV-related immune dysfunction may contribute to excess risk. We sought to assess whether sustained antiretroviral treatment (ART) reduces excess risk of anogenital cancer in PWH.

Methods: We conducted a case-cohort study in Botswana involving citizens aged 20 to 65. Adults with anogenital cancer were prospectively enrolled in a cohort ("Thabatse") from 2012-2020 at the 4 principal cancer treatment centers in Botswana. The subcohort was drawn from the Ya Tsie trial, which included a random 20% household sample of 30 communities (2013-2015) and an 80% sample in 6 communities (2017) in Botswana. ART duration was divided into three categories: No ART, ART < 5 years, and ART ≥ 5 years. We estimated the marginal relative risk (compared to HIV-uninfected) of incident anogenital cancer by ART duration using G-computation with inverse probability of treatment weights (IPTW), accounting for common factors of HPV and HIV, ART duration, and access to cancer treatment: 5-year age strata, education, age at first intercourse, smoking status, geographic region, and time period

Results: A total of 17,321 participants were enrolled, including 1,377 cancer cases. HIV prevalence was higher in individuals with cancer (80%) than persons without cancer (30%). PWH who received longer duration ART (≥ 5 years) were older than PWH who received shorter duration ART (< 5 years) (median age 44 and 38, respectively). Proportions of PWH on ART with CD4 nadir greater than 350 cells/µL were 55% and 58%, respectively. Greater than 95% of participants and parameters of access to cancer treatment were similar across groups (SMD <0.05 for included factors, <0.15). In all categories of ART duration, HIV-infection was associated with a large increase in risk for each cancer studied. Compared with shorter duration, longer duration ART was associated with greater risk of anogenital cancers: anus RR 1.86 (95% CI 1.2, 2.8); vulva RR 1.83 (95% CI 1.4, 2.5); penis RR 2.85, (95% CI 1.8, 3.7); cervix RR 1.12 (95% CI 1.0, 1.3, non-significant).

Conclusion: Prolonged ART does not reduce age-standardized anogenital cancer risk. Further interventions are needed to address excess cancer risk in PWH on ART.

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763 Trial Comparing Cryotherapy to LEEP in Women With HIV: CIN2+ Recurrence After 9 Years
Douglas K. Gaithe1, Marleen Temmerman1, Evans Nyongesa-Malava2, Shahin Sayed1, Stephen Hawes1, Andrew Nagy3, Samah Sakr4, Judith Lukorton5, Aisha Bwanaali1, Dennis Omordi1, Nelly R. Rugii1, James Kiarie1, Michael Chung6, Carey Farquhar7, Christine McGrath8
1Agha Khan University, Nairobi, Kenya, 2Coptic Hospital, Nairobi, Kenya, 3University of Washington, Seattle, WA, USA, 4Coptic Hope Centre, Nairobi, Kenya, 5Kenya Medical Research Institute, Nairobi, Kenya, 6University of Nairobi, Nairobi, Kenya, 7Emory University, Atlanta, GA, USA

Background: We aimed to determine the long-term risk of recurrent cervical intraepithelial neoplasia grade 2 or higher (CIN2+) in women living with HIV (WLWH) previously treated for cervical disease with loop electrosurgical excision procedure (LEEP) or cryotherapy.

Methods: We conducted a long-term follow-up study of WLWH, and CIN2/3 randomized to LEEP or cryotherapy and followed 2 years post-treatment in Kenya between 2011-2016. Former participants were recontacted from January to August 2023 for Papanicolaou (PAP) screening and colposcopy-directed biopsy if indicated to determine presence of CIN2+ in ≥ 9 years since initial treatment. Women with PAP results of low grade squamous intraepithelial lesions, high grade squamous intraepithelial lesions (HSIL) or Atypical Squamous Cells, HSIL cannot be excluded (ASC-H), underwent colposcopy-directed biopsy. Primary outcome was CIN2+ recurrence (CIN2/3, Carcinoma in-situ or invasive cancer) as determined by colposcopy-directed biopsy at ≥ 9 years after initial treatment. Log-binomial regression was used to estimate the relative risk of CIN2+ recurrence by treatment arm.

Results: Overall, 353 of 400 (88%) former participants were alive at the end of the parent trial, 14 were lost to follow-up with 33 mortalities. Of these, 288 (82%) were recontacted and 213 (74%) agreed to participate in this follow-up study. Fewer women in the LEEP arm (17%) had unsuppressed HIV viral load (≥1000 copies/ml) than the cryotherapy arm (24%). ART duration was similar between arms with a median of 11 years (IQR 10–12). Sixteen (8%) participants had a hysterectomy since the parent trial and were excluded. Of 197 screened, 5 (3%) of 107 in the cryotherapy arm and 3 (3%) of 90 in the LEEP arm had recurrent CIN2+ at ≥ 9 years since initial treatment. Of these eight with CIN2+, 2 (personal) had a prior CIN2+ recurrence in the 2 years following initial treatment (parent trial). In addition, 48 (31 in cryotherapy and 17 in LEEP) had CIN2+ recurrence in the initial trial but not in this study. There was no difference in CIN2+ recurrence risk at ≥ 9 years following initial treatment between arms (RR=1.40, 95% CI, 0.34-5.71, p=0.64). In subgroup analysis, the effect of treatment on CIN2+ recurrence did not vary by viral suppression, CD4 count, and ART duration.

Conclusion: Recurrence of CIN2+ in WLWH ≥ 9 years after cryotherapy or LEEP was low (4%) and did not differ between the ablative and excisional therapies after initial 2-year follow-up and may be related to long-term ART use.

764 Prioritizing Anal Cancer Screening in PWH: Not All Are at the Same Risk
Raquel Martin-Igucel1, Boris Rivollo2, Jordi Azcuita1, Pedro Domingo3, Joaquín Burgos4, Patricia Sorri5, María Saumoy6, Hernando Knobel3, Marta Navarro3, Elena Leon7, Amat Orts8, José M. Míni9, Jordi Casabona10, Josep M. Llibre11, for the PISCIS Cohort Study Group1
1Centre d’Estudis Epidemiològics Sobre les ITS i Sida de Catalunya, Barcelona, Spain, 2Hospital Germans Trias i Pujol, Barcelona, Spain, 3Hospital Sant Pau, Barcelona, Spain, 4Hospital Universitario de la Vall d’Hebron, Barcelona, Spain, 5Hospital Son Llàtzer, Palma de Mallorca, Spain, 6Hospital Universitario de Bellvitge, Barcelona, Spain, 7Hospital del Mar, Barcelona, Spain, 8Parc, Saúl Hospital Universitari, Sabadell, Spain, 9Hospital Moises Broggi, Sant Joan Despí, Spain, 10Verge de la Cinta Hospital, Tortosa, Spain, 11Hospital Clinic of Barcelona, Barcelona, Spain

Background: People with HIV (PWH) are up to 100 times more likely to develop anal cancer (AC) compared to the general population, where overall incidence is 1.6/100,000 person-years (PY). Screening programs are efficient in preventing AC but are not accessible to all PWH. Identifying individuals at higher risk is crucial to implement effective and targeted screening strategies.

Methods: In this cohort study, we included all treatment naïve PWH ≥16 years from 16 hospitals in Catalonia and Balearic Islands, included in the PISCIS HIV cohort from 1998-2022. The primary outcome was the incidence rate (IR) of histologically confirmed AC. We used Poisson regression to identify AC risk factors, including age at AC, risk group, nadir CD4+ count, and period of HIV diagnosis. AC and nadir CD4+ were validated in all cases.

Results: We identified 107 AC events among 14,238 PWH, overall IR of 71.5/100,000 PY (95% CI 71.5-71.6), and median follow-up 9.5 years (IQR 4.4-15.7). Of them, 37 died, 65% AC-related deaths. The IR was highest in those with nadir CD4+ <200 cells/µL (103.0 [95%CI:102.9-103.1]), followed by those with
Association Between HIV Infection and Survival for Head and Neck Cancers in the US From 2008-2020

Devesh S. Malgave, Ado S. Rivera, Christine Hartman, Jennifer R. Kramer, Peter Richardson1, Eftalicia Zaferioupolou2, Ruilin Hechter3, Matthew Boyer4, Dong Yongquan5, Lori Sakoda6, Donna White7, Wendy Leyden8, Michael J. Silverberg9, Elizabeth Y. Chiao10

1University of Texas at Houston, Houston, TX, USA, 2Kaiser Permanente Southern California, Pasadena, CA, USA, 3Baylor College of Medicine, Houston, TX, USA, 4Duke University School of Medicine, Durham, NC, USA, 5Kaiser Permanente Northern California, Oakland, CA, USA, 6University of Texas MD Anderson Cancer Center, Houston, TX, USA

Background: People with HIV (PWH) are at an increased risk of head and neck cancer (HNC) compared with people without HIV (PWoH). However, little is known regarding the impact of HIV on HNC survival. This study is one of the first to compare survival, by histological subtype of HNC, between PWH and PWoH.

Methods: A retrospective cohort of adults with newly diagnosed 1) oropharyngeal, 2)non-oropharyngeal (hypopharynx and larynx), 3) oral cavity cancers was identified using electronic health record data from the National Veterans Administration and Kaiser Permanente California regions from 2008 to 2020. Separate Cox regression models were used to assess the association of HIV infection with overall survival (OS) for each cancer type, adjusting for baseline sociodemographic factors, clinical characteristics, and HPV+ tumor status (oropharyngeal only). Multiple imputation was used for missing covariate data.

Results: Our analysis included 300 PWH and 39,950 PWoH HNC patients. Compared with PWoH, PWH were more likely to be younger; male; Black or Hispanic; had lower education attainment and income levels; and have co-infection with HPV, HBC, or HCV. Mortality rates for PWH vs. PWoH were 13.0 vs.9.7 per 100 person-years for oropharyngeal; 7.3 vs.13.0 for oral cavity; and 12.0 vs.14.0 for non-oropharyngeal cancers. In unadjusted analysis, HIV infection was associated with worse OS for oropharyngeal cancer (hazard ratio [HR]:1.31, 95%CI:1.02-1.66); better OS for oral cavity cancer (HR:0.57, 95%CI:0.39-0.82); and similar OS for non-oropharyngeal cancers (HR:0.83,95%CI:0.63-1.10). In adjusted analysis, similar trends were observed with worse OS in oropharyngeal cancer patients (HR:1.38, 95%CI:1.08-1.77); better OS in oral cavity cancer (HR:0.66, 95%CI:0.46-0.96); and similar OS for non-oropharyngeal cancer (HR:0.94, 95%CI:0.71-1.25). However, for oropharyngeal cancers, patients with HPV+ tumors had better survival compared with patients with HPV-negative tumors (HR:0.50, 95%CI:0.47-0.53). Other risk factors for poor OS for HNCs included older age at cancer diagnosis, early year of cancer diagnosis, higher stage of cancer, higher Charlson comorbidity index, alcohol abuse diagnosis, and ever smoking.

Conclusion: The study results suggest that PWH had poor survival for oropharyngeal cancer but better survival for oral cavity cancers compared with PWoH. Further research should focus on finding the etiological factors influencing HIV’s role in outcomes related to HNCs.

Table 1. Descriptive statistics of head and neck cancer patients who received healthcare at the national veterans administration and Kaiser Permanente California regions from 2008-2020 stratified by HIV status.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Veterans Administration (N=228)</th>
<th>PWoH (N=7742)</th>
<th>PWH (N=2172)</th>
<th>Total (N=8190)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer histologic subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oropharyngeal</td>
<td>96 (42.0)</td>
<td>9760 (20.2)</td>
<td>8900 (20.5)</td>
<td>3779 (47.3)</td>
<td>0.38</td>
</tr>
<tr>
<td>Non-Oropharyngeal</td>
<td>80 (36.8)</td>
<td>7570 (16.7)</td>
<td>613 (14.4)</td>
<td>1464 (18.8)</td>
<td>0.38</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>49 (21.4)</td>
<td>550 (0.3)</td>
<td>116 (2.7)</td>
<td>132 (0.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>Head and neck</td>
<td>143 (64.0)</td>
<td>9896 (22.2)</td>
<td>1006 (23.9)</td>
<td>2039 (26.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>HCV</td>
<td>11 (5.3)</td>
<td>158 (0.3)</td>
<td>15 (0.3)</td>
<td>12 (0.2)</td>
<td>0.02</td>
</tr>
<tr>
<td>HBC</td>
<td>11 (5.3)</td>
<td>158 (0.3)</td>
<td>15 (0.3)</td>
<td>12 (0.2)</td>
<td>0.02</td>
</tr>
<tr>
<td>Death within the first year</td>
<td>57 (25.4)</td>
<td>4662 (10.9)</td>
<td>573 (13.0)</td>
<td>596 (7.3)</td>
<td>0.66</td>
</tr>
</tbody>
</table>


Changes in Gut Microbiota Associated With Progression of Atherosclerosis in People With HIV

Javier García-Abellán1, Marta Fernández-González2, Vanesa Aguillo1, José Alberto García1, Sergio Padilla1, Catalina Robledano1, Ronaldo Gandámez2, Leandro López1, Paula Mascarell1, Angelina Botella1, Nuria Ena1, Lidia García1, María José González2, Félix Gutiérrez1, Mar Masiá3

1Hospital General Universitario de Elche, Elche, Spain, 2FASBIO, Valencia, Spain

Background: Variations in gut microbiome might modulate host immune activation and inflammation, potentially linked to increased cardiovascular risk. Our objective was to longitudinally characterize the distinctive features of gut microbiota associated with progression of atherosclerosis measured through carotid artery intima-media thickness (cIMT) in people living with HIV (PLWH).

Methods: 96-week, longitudinal study in virologically suppressed (HIV-1 RNA <50 copies/mL) PLWH with no previous cardiovascular disease. cIMT was

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Contribution of HIV to the Burden of Merkel Cell Carcinoma in the US

Jacob T. Tribble1, Karena Volsky-Avellaneda2, Qianlai Luo1, Brenda Hernandez2, Eric Engels3

1National Cancer Institute, Rockville, MD, USA, 2National Institutes of Health, Bethesda, MD, USA, 3Texas Department of State Health Services, Austin, TX, USA, 4University of Hawaii, Honolulu, HI, USA

Background: Merkel cell carcinoma (MCC) is an aggressive form of skin cancer with a 5-year survival rate of approximately 65%. Risk factors for MCC include advanced age, immunosuppression, Merkel cell polyomavirus, lightly pigmented skin, and UV radiation. People with HIV (PWH) have elevated risk of MCC; however, prior studies had few MCC patients with HIV (ranging from 6 to 20) and did not estimate the MCC burden attributable to HIV. Using population-based cancer registry data collected in 14 US regions, we report the characteristics of MCC patients with HIV and quantify the burden of MCC among PWH during 2001–2019.

Methods: We used cancer registries in the HIV/AIDS Cancer Match (HACM) Study to identify MCC (ICD-0-0) between 2001–2019. The linked HIV registry data to compare cases in PWH and those without HIV. We calculated standardized incidence ratios (SIRs) to compare MCC incidence in PWH to the general population. To estimate the proportion of MCC cases in the general population due to HIV, we calculated population attributable fractions (PAFs) using the formula Pct.*(IRR-1)/RR, where Pct is the prevalence of HIV among MCC patients and RR is the relative risk of MCC associated with HIV (approximated by the SIR). We also report HIV viral load and CD4 counts among PWH in the year leading up to MCC diagnosis.

Results: 13,262 MCC cases were diagnosed in HACM registries during 2001–2019, and 46 (0.4%) were among PWH (see table). Among MCC patients, the median age of diagnosis was 56 years (IQR:50–67) in PWH, vs. 77 years (IQR: 68–84) in those without HIV. Thirty-one (67%) PWH and MCC had an AIDS diagnosis prior to MCC diagnosis. The risk of PWH developing MCC was almost triple the risk of MCC diagnosis prior to MCC diagnosis. With MCC developing prior to MCC diagnosis. The risk of PWH developing MCC was almost triple the risk of MCC diagnosis prior to MCC diagnosis. The risk of PWH developing MCC was almost triple the risk of MCC diagnosis prior to MCC diagnosis. The risk of PWH developing MCC was almost triple the risk of MCC diagnosis prior to MCC diagnosis. The risk of PWH developing MCC was almost triple the risk of MCC diagnosis prior to MCC diagnosis. The risk of PWH developing MCC was almost triple the risk of MCC diagnosis prior to MCC diagnosis.

Conclusion: PWH have a higher risk of MCC compared to the general population. While the overall contribution of HIV to the MCC burden is minor, a larger PAF was observed among younger individuals. The majority of PWH and MCC had disease markers indicating moderate to severe immunosuppression leading up to MCC diagnosis.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PWH with HIV/AIDS</th>
<th>PWH without HIV/AIDS</th>
<th>PWH-VAH</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>46 (42.5%)</td>
<td>12,000 (20.1%)</td>
<td>2790 (20.8%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Sec. IIB</td>
<td>41 (85.1%)</td>
<td>8595 (42.2%)</td>
<td>2051 (21.2%)</td>
<td>0.3</td>
</tr>
<tr>
<td>20 to 59 years old</td>
<td>30 (69.0%)</td>
<td>1050 (50.9%)</td>
<td>2160 (21.9%)</td>
<td>0.2</td>
</tr>
<tr>
<td>60+ years old</td>
<td>16 (39.5%)</td>
<td>1130 (53.2%)</td>
<td>630 (6.3%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>21 (52.4%)</td>
<td>6195 (30.1%)</td>
<td>1820 (19.0%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>4 (10.7%)</td>
<td>212 (1.1%)</td>
<td>17 (1.8%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>34 (87.2%)</td>
<td>11,893 (59.1%)</td>
<td>593 (6.2%)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* MCC cases and HIV-1 RNA <50 copies/mL were counted at time of diagnosis and linked to the case in the registry.
measured at baseline, 48 and 96-weeks visits using B-mode ultrasound at 6 locations. CIMT progression was defined as an increase >10% and/or detection of new carotid plaques. To profile the gut microbiome, DNA was extracted from fecal samples and quantified with the Qubit dsDNA HS Assay Kit. Amplification and sequencing of 16S ribosomal RNA (V3-V4 variable regions) were carried out following the Illumina protocol. For bioinformatic analysis of amplicons, the QIIME package was used. Biomarker analysis was performed using the LEFSe software package.

Results: 202, 190 and 169 patients had available fecal samples for microbiome analysis at the baseline, 48- and 96-week visits, respectively. At the 96w visit, 87 (43%) patients were categorized as progressed, with SA (26.7%) of them showing a new carotid plaque. No significant differences were observed in α-diversity indices between groups at baseline. Over the 96w follow-up period, a decrease in α-diversity (Simpson’s and Shannon’s indices) was observed in the progressor group. Based on beta-diversity analysis at the species level, determined through principal coordinate analysis (PCoA) distances, the progressor and non-progressor groups exhibited distinct and divergent microbial profiles during each follow-up visit (p=0.001). When all sequences from the three visits were compared, the PCoA plots of Bray-Curtis distance revealed that the samples clustered in accordance with the progression of atherosclerosis (p=0.016; Permanova test) (Fig1A). LEFSe analysis revealed distinct associations between progression and specific bacterial taxa (Fig1B).

Agathobacter, Ruminococcus_2 and Lachnospiraceae_UCG_003 genus were consistently observed in various visits as distinctive genuses associated with progression, while Prevotella_7 and Allisonella genus were linked to non-progressor patients.

Conclusion: Progression of atherosclerosis in PLWH is associated with distinctive changes in the gut microbiota.

Plasma IL-1β Predicts Incident Coronary Artery Disease and Pulmonary Embolism in PWH on ART

Sannidhi Sarvadhavahalli1, Lei Shi1, Junho Cho2, Maria Sophia B. Donaire2, Vivian Pae3, Alton Barbehenet, Danny Li4, Xiuping Chui5, Colin T. Maguire4, Priscilla Y. Huse6, Jingshen Wang7, Rafiq P. Sekaly1, Brian K. Agan8, Jeffrey A. Tomai8, Sulagia A. Lee8

1University of California San Francisco, San Francisco, CA, USA, 2University of California Berkeley, Berkeley, CA, USA, 3Infectious Disease Clinical Research Program, Bethesda, MD, USA, 4University of Utah, Salt Lake City, UT, USA, 5Emory University, Atlanta, GA, USA

Background: Despite antiretroviral therapy (ART), people with HIV (PWH) experience higher rates of morbidity and mortality vs. age-matched HIV-negative controls which may be driven by chronic inflammation due to persistent virus. Plasma interleukin 6 (IL-6) levels are amongst the strongest predictors of mortality in PWH on ART, and an upstream regulator of IL-6, interleukin-1 beta (IL-1β), may be the major driver of this risk.

Methods: We performed a case-cohort study of 1,002 PWH on ART from the U.S. Military HIV Natural History Study. Inclusion criteria were HIV-1 infection and plasma HIV RNA <50 copies/ml. Cases were PWH on ART with incident vascular event (VE): coronary artery disease (CAD), CAD with myocardial infarction (MI), deep vein thrombosis (DVT), pulmonary embolus (PE), cerebrovascular accident (CVA, stroke), and/or peripheral arterial disease (PAD). Cases were age- and gender-matched 1:4 to controls. Cryopreserved plasma (>1 year prior to censor date) were used to quantify IL-1β signaling cytokines (IL-1β, IL-6, and IL-18) using a high-sensitivity assay (MesoScale). Logistic regression models adjusted for potential vascular covariates (hypertension, hyperlipidemia, diabetes mellitus, tobacco use, alcohol use, family history of vascular disease) were fit to estimate odds ratios (ORs).

Results: A total of 200 cases and 802 controls were included. Participants were mostly male (92%), White (43%; 41% Black, 10% Hispanic), with a median age=31 years, nadir CD4= T cell count=200 cells/mm³, pre-ART viral load=log10 4.6 copies/mL, ART suppression=6.8 years, and HIV seroconversion to ART initiation=1.8 years; overall characteristics were similar between cases vs. controls. Plasma IL-1β, but not IL-18 or IL-6, significantly predicted the composite outcome of any vascular event (OR, VE=1.21, 95% CI:1.10, 1.33) and predicted CAD, MI, and PE. The associations with PE and CAD remained significant in multivariate models adjusted for hypertension and hyperlipidemia (OR_PE=1.38, 95% CI: 1.09, 1.73 and OR_CAD=1.21, 95% CI: 1.07, 1.38).

Conclusion: Recent high sensitivity assays can now discriminate within- and across-individual plasma IL-1β levels. To our knowledge, this is the largest epidemiologic study of plasma IL-1β in PWH, as well as in the general population, in relation to vascular events. Our findings suggest that IL-1β may be a critical upstream regulator of IL-6 and have important implications for therapeutic strategies to reduce inflammation and vascular comorbidities in PWH on ART.

Clonal Hematopoiesis in HIV and Atherosclerosis, Arterial Inflammation, and Hematopoietic Activity

Matthew S. Durstenfeld9, Katherine J. Kentooff10, Alexandra J. Teng11, Shady Abouhashem12, Danny Li13, Rebecca Hoi13, Steven G. Deeks13, Alexander G. Bick11, Ahmed A. Tawakol14, Priscilla Y. Huse13

1University of California San Francisco, San Francisco, CA, USA, 2Beth Israel Deaconess Medical Center, Boston, MA, USA, 3Kaiser Permanente, Oakland, CA, USA, 4Massachusetts General Hospital, Boston, MA, USA, 5Vanderbilt University, Nashville, TN, USA

Background: Clonal hematopoiesis of indeterminate potential (CHIP) is associated with cardiovascular disease (CVD) and common in HIV, but whether CHIP contributes to atherosclerosis in HIV is unknown. We hypothesized that CHIP is associated with atherosclerosis, arterial inflammation, and hematopoietic activity among people with HIV (PWH).

Methods: In this observational study, we studied treated, suppressed PWH ages 35-70 years old with ≥1 CVD risk factor. CHIP mutations were detected from peripheral blood mononuclear cells with a validated targeted sequencing assay. We measured carotid intima-media thickness (IMT) longitudinally with ultrasound and aortic inflammation and hematopoietic metabolic activity using cross-sectional 18F-FDG-PET. Inflammatory biomarkers were measured with a multiplex electrochemiluminescence assay. We used linear regression with adjustment for age, sex, nadir CD4 count, smoking, hypertension, diabetes, and hyperlipidemia to adjust for potential confounders.

Results: We included 231 PWH (52±9 years, 7% female), 32 (14%) had CHIP with median variant allele fraction of 0.3%. Common mutations were in DNMT3A (n=21) and TET2 (n=6). Only age was associated with CHIP (OR 2.3 per decade older, 95%CI 1.2-3.9; p=0.003. Among N=165 (CHIP=22), baseline mean IMT was 1.0 mm with and without CHIP (p=0.63), unchanged after adjustment (Figure). CHIP was not associated with prevalent (p=0.82) or incident plaque (p=0.48). Over 3.2 years, IMT progression was faster among those with CHIP (0.033 mm/year; p=0.01), attenuated after adjustment (0.022 mm/year;
Activated and Exhausted CD8+ T-Cell Subsets Associate With Carotid Atherosclerotic Plaques in PLHIV

Marc Blaauw1, Adriana Navas1, Nadia Vadaq1, Vasiliki Matzaraki1, Elise M. Meeder1, Wilhelm A. Vos1, Albert L. Groenendijk1, Louise E. van Eekeren1, Gert Weijers1, Marvin Berrevoets1, Hans Koenen1, Marien I. de Jonge1, André J. van der Ven1, Joost Rutten1, Niels P. Riksen1

Rotterdam University Medical Center, Nijmegen, Netherlands, 1Eunicea University Medical Center, Rotterdam, Netherlands, 1Elisabeth-TweeSteden Ziekenhuis, Tilburg, Netherlands

Background: Atherosclerotic cardiovascular disease (ASCVD) is one of the leading causes of morbidity and mortality in people living with HIV (PLHIV). Understanding the association between circulating immune cells and carotid plaque presence in PLHIV holds the potential to identify novel immunological mechanisms driving plaque progression in PLHIV. Flow cytometry, allows identification and quantification of immune cells within heterogeneous populations.

Methods: Our study analyzed data from the 2000HIV study (NCT03994835), comprising 1188 individuals with valid carotid ultrasound, without previous ASCVD and with valid high-dimensional flow cytometry measurements. This Dutch multi-center cross-sectional study focuses on virally suppressed PLHIV under suppressive ART, we performed linear regression models adjusted for age, sex and seasonality. High dimensional flow cytometry was performed to identify the major blood immune cell subsets (403 populations) and their functional status. We identified differences within CD8+ T cell populations in individuals with carotid plaques as compared with individuals without carotid plaques.

Conclusion: Among PLHIV, CHI mutations were not associated with subclinical atherosclerosis or arterial inflammation, proposed mechanisms of how CHI could cause CVD. Associations with lymph node and spleen activity suggests CHI may be induced by HIV reservoirs or induce increased reservoir activity.

Clinical outcomes studies are needed to ascertain the impact of CHI on CVD in HIV.

771 Co-Occurrence of MASLD and CKD Increased Cardiovascular Incidence Among Thai HIV Population

Hay Mar Su Lwin1, Tanakorn Apornpong1, Win Min Hani2, Sivapon Gatechompai1, Siwat Thammapriwan1, Pornpep Amornvitanch1, Stephen Kerr1, Anucha Avihingsanon1

1Thai Red Cross AIDS Research Center, Bangkok, Thailand, 2Kirby Institute, Sydney, Australia, 3Chulalongkorn University, Bangkok, Thailand

Background: Combination of Metabolic dysfunction Associated Steatotic Liver Disease (MASLD) and chronic kidney disease (CKD) are associated with cardiovascular diseases (CVDs). However, this importance finding in people living with HIV (PWH) is largely unknown. We therefore investigated the effect of combined diagnosis of MASLD and CKD on the incidence of CVDs among PWH in Thailand.

Methods: All PWH starting ART in a prospective cohort with available transient elastography (controlled attenuation parameter (CAP)) and serum creatinine during June 2013 to Jul 2023 were included. MASLD was defined as steatotic liver disease (CAP ≥ 248 dB/m) plus one of five cardiometabolic criteria: body mass index (BMI) ≥ 25kg/m² or waist circumference > 94cm for males and > 80cm for females; fasting glucose ≥ 100mg/dL or a diagnosis of diabetes mellitus; BP ≥ 130/85mmHg or a diagnosis of hypertension; triglyceride ≥ 150mg/dL or lipid lowering agents, and HLD ≤ 40mg/dL or females or ≤ 50mg/dL for females. CKD was defined as estimated glomerular filtration rate (eGFR) ≤ 60ml/min/1.73m² using the CKD Epidemiology Collaboration. CVDs included coronary diseases, stroke, peripheral arterial disease and aortic disease. Follow-up started at the 1st fibroscan/eGFR measurement and ended at the first cardiovascular disease diagnosis or death. Multivariable Cox regression adjusting for potential confounders was used to evaluate factors associated with CVDs development, adjusted for potential confounders.

Results: Totally 1940 PWH (70.8% male, median age 43 [interquartile range (IQR): 32-50] years; BMI 22.5 [IQR: 20.6-24.9]; current smoker 18% (n=265); abacavir exposure 7.5% (n=143) were analysed. The prevalence of MASLD+/CKD+ at baseline was 6.4% (n=125). During a median 4.1 years of follow-up, 20 participants developed new-onset CVDs. Multivariable Cox regression adjustment for age, sex, smoking, ART duration and protease inhibitors use showed that PWH with a combined diagnosis of MASLD and CKD at baseline was associated with a significantly increased risk of developing CVD (adjusted hazard ratio [aHR] = 7.9 (95%CI 2.5-24.7), P<0.001), but the presence of either comorbidity alone did not significantly increase the risk [aHR = 1.04 (95%CI 0.3 – 3.7); P=0.96] (Figure).

Conclusion: MASLD together with CKD was associated with a significantly increased risk of new-onset CVDs, but MASLD or CKD alone was not. Further studies to understand the mechanisms for the increased risk are warranted for the prevention of CVDs in this group.
772 The Association of Mild Kidney Disease With Coronary Disease Is Stronger for People With HIV
Shradha J. Doshi1, Barbra Richardson2, Rashidah Nazzinda1, Henry Mugerwa3, Marcio Bittencourt4, Geoffrey Erem2, Apana Narendra4, Carey Farquhar5, Cissy Kityo6, Chris T. Longenecker2
1University of Washington, Nairobi, Kenya, 2University of Washington, Seattle, WA, USA, 3Joint Clinical Research Centre, Kampala, Uganda, 4University of Pittsburgh, Pittsburgh, PA, USA, 5St. Francis Hospital Noaanya, Kampala, Uganda, 6New York University, New York, NY, USA

Background: Chronic kidney disease (CKD)—a common comorbidity for people with HIV (PWH)—is also a risk factor for coronary artery disease (CAD). However, the impact of mildly impaired eGFR and microalbuminuria on CAD risk for PWH is not well defined. Using coronary computed tomography angiography (CCTA), we examined whether the association between mild kidney disease and coronary plaque might be stronger for PWH compared to people without HIV in sub-Saharan Africa.

Methods: We conducted a cross-sectional secondary analysis of data from the Ugandan study of HIV effects on the myocardium and atherosclerosis (mUTIMA) study. mUTIMA compared 100 PWH on stable antiretroviral therapy (ART) and 100 age- and sex-matched participants without HIV, all aged over 45 with ≥1 cardiovascular disease risk factor. Participants with screening eGFR <60 mL/min/1.73 m² were excluded due to risk of contrast-induced nephropathy. For 165 of these participants with available CCTA data, we performed multivariable Tobit regression stratified by HIV status to examine the association of CAD with both estimated glomerular filtration rate (eGFR) and albumin creatinine ratio (ACR). CAD was characterized using segment involvement score (SIS; total segments with plaque), segment stenosis score (SSS; segment score weighted by degree of luminal obstruction), and coronary artery calcium (CAC) score.

Results: Mean (SD) age was 57.7(6.9) years and 62.4% were female. Mean (SD) systolic blood pressure was 152.2(27.8) mmHg and 31.5% had diabetes. 96% had HIV-1 viral suppression and 47% were on tenofovir (TDF). Among PWH—but not among participants without HIV—mildly impaired eGFR (<90 mL/min/1.73 m²) was associated with higher SIS (β 3.31, 95% CI: 0.41 to 6.21, p = 0.03) and SSS (β 5.95, 95% CI: 0.54 to 11.36, p = 0.03). The association with SIS remained significant after adjusting for age, gender, and 10-year ASCVD score (β 2.58, 95% CI: 0.10 to 5.06, p = 0.04). Associations of ACR with coronary plaque were not statistically significant for participants with or without HIV (all p > 0.07).

Conclusion: Mild to moderately low eGFR is more strongly associated with CAD in PWH compared to those without HIV in Uganda. In addition to assessing traditional risk factors, monitoring renal function may add to cardiovascular risk assessment of PWH in sub-Saharan Africa. The observed association also highlights the need to integrate cardiovascular and renal care into HIV clinics, especially for aging PWH with higher rates of comorbid diabetes and hypertension.

Table: Relationship between eGFR, ACR, and segment involvement score (SIS) in PWH and HIV-negative controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV negative (n=105)</th>
<th>HIV positive (n=105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>&lt;0.07</td>
<td>-2.3(2.25)</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>&lt;0.001</td>
<td>0.22</td>
</tr>
</tbody>
</table>

773 Related Pitavastatin Effects on Inflammatory Biomarkers to Plaque Changes in REPRIEVE
Steven Grinspoon1, Michael T. Lu,2, Sara McCallum3, Markella Zanni4, Borek Foldyna5, Kayla Paradis6, Carl J. Fichtenbaum7, Judith A. Aber8, Gerald S. Bloomfield9, Carlos D. Malvestutto10, Judith S. Currier11, Thomas Mayrhofer12, Julia Karady13, Heather J. Ribaudo14, Pamela S. Douglas15
1Massachusetts General Hospital, Boston, MA, USA, 2University of Cincinnati, Cincinnati, OH, USA, 3Columbia School of Medicine at Mt Sinai, New York, NY, USA, 4Duke University, Durham, NC, USA, 5The Ohio State University, Columbus, OH, USA, 6University of California Los Angeles, Los Angeles, CA, USA, 7Harvard TH Chan School of Public Health, Boston, MA, USA, 8Duke University School of Medicine, Durham, NC, USA

Background: The REPRIEVE trial demonstrated that pitavastatin reduced the risk of major adverse cardiovascular events (MACE) by 35% and LDL by 30% compared to placebo over median 5.1 years of follow up. The REPRIEVE mechanistic study further demonstrated pitavastatin was associated with a significant reduction in progression of noncalcified coronary plaque volume compared to placebo as well as changes in oxLDL and LpPLA2. This analysis further interrogates these findings to examine whether pitavastatin effects on lipids and these inflammatory biomarkers relate to changes in plaque.

Methods: Coronary plaque volume was assessed at baseline and 24 months by CT angiography, using a threshold of <350 HU to define noncalcified plaque. Biomarker changes were analyzed on the log2 scale. Pearson correlation was used to assess associations between changes in LDL, biomarkers, and plaque (0 to 24 months). For this mechanistic assessment, analyses were restricted to participants who remained on randomized treatment for 24 months with biomarkers assessed.

Results: The 624 participants (304 pitavastatin, 300 placebo) included in the analysis were representative of the substudy population (mean age 51 years, sex 17% female, CVD risk median 4.6%). Over 24 months, pitavastatin was associated with a 19% [95% CI 8.9, 29] reduction in oxLDL and a 10% [-6, 14] reduction in LpPLA2 compared to neutral changes or increases in the placebo group. Corresponding mean fold changes in each group were 0.81 [0.71, 0.92] vs. 1.00 [0.89, 1.13] for oxLDL and 0.90 [0.87, 0.94] vs. 1.17 [1.13, 1.21] for LpPLA2, pitavastatin vs. placebo, respectively. No associations between changes in LDL, oxLDL, or LpPLA2, and either non-calcified or total plaque volume were apparent (ρ < 0.15, Figure). Adjustment for changes in the biomarkers did not impact the effect of pitavastatin to reduce noncalcified plaque. Similar results were seen in the entire substudy population and the subset with plaque at baseline.

Conclusion: Pitavastatin significantly reduced LDL and key biomarkers of lipid oxidation and arterial inflammation in REPRIEVE, but these changes were not associated with changes in noncalcified or total plaque volumes. Further studies will assess effects of these pathways on MACE and also the effects of pitavastatin on other mechanistic pathways which might link statin effects to improvements in coronary artery disease indices and MACE in PWH.

774 Endothelial Microvesicles: Circulating Biomarker and Mediator of Vascular Dysfunction With HIV-1
Emily I. Ostroff1, Vinicius P. Garcia1, Hannah K. Fandl1, Auburn R. Berry2, Hannah L. Gardena1, Jared J. Greiner1, Brian L. Stauffer2, Elizabeth Connick3, Christopher DeSouza1
1University of Colorado Boulder, Boulder, CO, USA, 2University of Arizona, Tucson, AZ, USA, 3Ohio State University, Columbus, OH, USA

Background: We have previously reported that circulating endothelial cell derived extracellular microvesicles (EMVs) are elevated in adults living with HIV (ALWH) receiving antiretroviral therapy (ART) contributing to endothelial vasodilator dysfunction by negatively affecting endothelial nitric oxide synthase (eNOS) and, in turn, impairing NO production. However, whether elevations in circulating EMVs and their pathologic effects on endothelial NO production was
a direct effect of HIV-1 or a consequence of ART is unknown. The experimental aims of this study were to determine: 1) whether circulating EMVs are elevated in treatment-naive ALWH and associated with HIV-1-related endothelial vasodilator dysfunction; and 2) the effects of EMVs isolated from treatment-naive ALWH on endothelial cell NO production, in vitro.

**Methods:** Twenty-four sedentary, adults were studied. 12 healthy (10M/2F; age 35±2 yr) and 12 treatment-naive ALWH (10M/2F; 35±2 yr; viral load: 7342 copies/mL). All subjects were non-obese, normotensive, nonobese, normotensive, and free of overt cardiometabolic disease. Circulating EMV (CD144-PE) number was determined by flow cytometry. Forearm blood flow (FBF; via plethysmography) was assessed by intraarterial infusion of acetylcholine and sodium nitroprusside. Human coronary artery endothelial cells were cultured and treated with EMVs (CD144-PE) isolated from the healthy and treatment naïve ALWH.

**Results:** Circulating EMVs were ~75% higher (P<0.05) in the treatment naïve ALWH (23±4.2 EMV/μL) vs healthy (13±3 EMV/μL) adults. FBF responses to acetylcholine were significantly lower (~20%) in the treatment naïve ALWH adults (from 5.6±0.3 to 12.6±1.1 mL/100 mL tissue/min vs 4.9±0.2 to 15.8±1.1 mL/100 mL tissue/min). There were no significant differences between the groups in FBF response to sodium nitroprusside. EMVs were strongly and inversely associated with the vasodilator response to acetylcholine (r=−0.49; P=0.01). Moreover, expression of phosphorylated eNOS (7.8±2.1 vs 10.2±7.9 AU) and NO production (6.0±0.4 vs 7.3±0.4 µmol/L) was significantly lower in cells treated with EMVs from treatment naïve ALWH vs healthy adults.

**Conclusion:** HIV-1 is associated with elevated circulating EMVs. Moreover, circulating EMVs are not only a systemic biomarker, but appear to be a mechanistic factor underlying endothelial dysfunction with HIV-1. Circulating EMVs represent a novel causative factor underlying vascular abnormalities and the increased CVD risk associated with HIV-1.

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**Endothelial Dysfunction in HIV: Exploring Cytokine and Chemokine Dynamics**

Celestine Wanjalla, Joey Stolze, Samuel Ballin, Mona Mashayekhi, Sheng Quanhu, Curtis L. Gabriel, Xiuxi Zhang, David G. Harrison, John Koethe

**Vanderbilt University, Nashville, TN, USA**

**Background:** In persons living with HIV (PWH), chronic inflammation promotes endothelial dysfunction, which contributes to cardiovascular disease. In this study, we sought to understand how subsets of endothelial cells (ECs) in adipose tissue modulate inflammation.

**Methods:** We analyzed the expression of cytokine/chemokine receptors by ECs in the subcutaneous adipose tissue of 59 PWH using single-cell RNA sequencing. We analyzed the association of EC subsets within adipose tissue with plasma cytokines and computed tomography morphometric measures. CellChat analysis was used to identify potential interactions between ECs and immune cells to further define the role of cytokines/chemokines. We also performed bulk RNA sequencing of human arterial ECs cultured in media conditioned with plasma from PWH to assess the direct effects of cytokines.

**Results:** The pattern of cytokine and chemokine receptor expression was different in the various subcutaneous adipose cell types identified: including capillary, venous, and arterial ECs; pericytes and vascular smooth muscle cells. Venous ECs expressed the ilr and the proportion of venous ECs was positively correlated with triglycerides (P=0.43, P<0.04), visceral fat volume (P=0.29, P=0.02), and plasma IL-6 (P=0.35, P<0.01), and negatively correlated with liver density (P=0.32, P=0.01). Capillary ECs had relatively low levels of ilr compared to venous endothelial cells and were positively correlated with the mean liver density (P=0.36, P<0.01) and negatively with visceral fat volume (P=-0.34, P=0.01), and plasma IL-6 (P=-0.29, P=0.01). Analysis of intercellular communication between ECs and immune cells shows that capillary ECs have the potential to secrete chemokines that signal directly to venous endothelial cells, and this is mediated by classical and non-classical monocytes (Figure A). Arterial ECs cultured with plasma from PWH when compared to media controls differentially expressed genes that enriched for the TNF-α pathway via NFKB, and reactive oxygen species pathway (Figure B).

**Conclusion:** Cytokine and chemokine signaling between subcutaneous adipose tissue arterial, capillary and venous endothelial cells is potentially mediated by classical and non-classical monocytes. In PWH, this may indicate a dynamic crosstalk between vascular and immune cells that may underly, in part, the increased risk of cardiometabolic conditions linked to chronic inflammation.
**Elevated Triglycerides and Endothelial Vasodilator Dysfunction in Adults Living With HIV-1**

Vinicius P. Garcia1, Kelly A. Stockelman1, Anthony R. Bain2, Cynthia Finhamber2, Jared J. Greiner1, Brian L. Stauffer1, Elizabeth Connick1, Christopher DeSouza1

1University of Colorado-Boulder, Boulder, CO, USA, 2University of Arizona, Tucson, AZ, USA

**Background:** Adults living with HIV (ALWH) commonly have high triglyceride (TG) levels contributing to their increased risk of cardiovascular disease (CVD). Indeed, elevated TG levels are independently associated with an increased risk of myocardial infarction in ALWH. A major underlying mechanism for the TG-related increase in CVD is a profound worsening in vascular endothelial function. The influence of high TG levels on vascular endothelial function in ALWH is unknown. We tested the hypotheses that: 1) high TG levels are associated with worse endothelium-dependent vasodilation in antiretroviral (ART)-treated ALWH; and 2) the TG-related reduction in endothelial vasodilator function is due, in part, to diminished nitric oxide (NO) bioavailability.

**Methods:** Forty-two sedentary, non-obese, normotensive, non-medicated adults (from ART) middle-aged men were studied: 14 healthy adults (age: 38±3 yr; TG: 73±8 mg/dL); 14 ALWH on stable antiretroviral therapy with normal TG (42±2 yr; 98±7 mg/dL); and 14 ALWH on stable antiretroviral therapy with elevated TG (43±2 yr; 234±17 mg/dL). Aside from TGs all other lipid and lipoproteins, glucose and insulin levels were clinically normal. Forearm blood flow (FBF) responses to intra-arterial infusion of the endothelial agonist acetylcholine (Ach), in the absence and presence of the endothelial NO synthase inhibitor NG-monomethyl-L-arginine (L-NMMA), as well as the endothelium-independent vasodilator sodium nitroprusside were determined by venous occlusion plethysmography.

**Results:** FBF responses to Ach were significantly lower (~20%) in the ALWH with normal TG (from 4.5±0.3 to 13.1±0.7 mL/100 mL tissue/min) vs healthy adults (4.9±0.3 to 17.3±0.7 mL/100 mL tissue/min); whereas, the ALWH with high TG (4.3±0.3 to 10.2±1.0 mL/100 mL tissue/min) demonstrated FBF responses to Ach significantly lower than ALWH with normal TG and healthy adults. L-NMMA significantly reduced the FBF response to Ach in the healthy (~30%) and ALWH with normal TG (~25%) but not the ALWH with high TG (~10%). There were no significant group differences in the FBF response to sodium nitroprusside. FBF levels were inversely and significantly associated with the FBF response to Ach (r=−0.51; P=0.003) in the ALWH.

**Conclusion:** Elevated TG levels is associated with lower endothelium-dependent vasodilation in adults living with HIV-1 due, in part, to reduced NO bioavailability. Diminished NO-mediated endothelium-dependent vasodilation may underlie the increased risk of CVD in ALWH with high TG levels.

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**Spatial Profiling Reveals Immunometabolic Pathways Involved in Trained Immunity in PWH With CVD**

Cheryl Cameron1, Emily Bowman1, Leah Zagore1, Banumathi Tamilselvan1, Mohammad-Ali Jenabian1, Jean-Pierre Routy1, Madeleine Durand2, Remi Bunet1, David P. Maison4, Arianne Caudal1, Michael M. Lederman2, Elizabeth Mayne2, Aurelie Cleret-Buhot1, Cynthia Firnhaber1, James Salazar1, Brian L. Stauffer1, Jared J. Jon Lomasney3, Christopher DeSouza3, Leah Zagore2, Réjean Thomas1, Timothy J. Kelly A. Stockelman3, 4

1University of Arizona, Tucson, AZ, USA, 2The Ohio State University, Columbus, OH, USA, 3The Ohio State University, Columbus, OH, USA, 4University of Cape Town, Cape Town, South Africa

**Background:** People with HIV (PWH) are at increased risk for cardiovascular disease (CVD). Chronic exposure of innate immune cells to microbial products and pro-inflammatory lipids may drive trained immunity in these cells through transcriptional and metabolic changes.

**Methods:** We measured coronary artery calcium (CAC) scores among people with and without HIV (N=25,43) to assess atherosclerosis and vascular age. Blood was collected from participants for assay of serum markers of immune activation and differentiation of monocyte-derived macrophages (MDMs). We assessed the gene expression by RNAseq and cellular metabolism of MDMs (Seahorse) and responsiveness of these cells to bacterial products (LPS, Pam3CysSerLys4, or flagellin). We performed spatial profiling of arterial blood vessels from PWH and people without HIV (PWH) using NanoString’s GeoMx.

**Results:** PWH had increased CAC scores (350 vs 18: P=0.04) compared to PWH, and among PWH vascular ages were higher than their biological ages (77± vs 55±: P=0.0001). Increased CAC was associated with elevated inflammatory markers (osteopontin, C-reactive protein, IL-6, soluble CD14: P=0.02). MDMs from PWH had altered gene expression in response to bacterial products, including higher HIF1α, mTOR, AKT1, MyD88 and NFκB, and were more dependent on glycolysis, all hallmarks of innate trained immunity. Spatial profiling confirmed increased macrophage infiltration into vessel walls in PWH. Pathway analysis of coronary arteries from PWH vs PWH showed increased enrichment of glycolysis, TLR signaling, AKT, JNK and MTOR pathways. A cross-sectional comparison of regions of interest (ROI) with highest infiltration showed TLR4 and CD163 was higher in PWH. In ROI with lower infiltration, higher expression of FABP12 and CD163 was found in tissues from PWH. Analysis of pathways driving higher infiltration in both donor types identified unique pathways in PWH including innate immunity, TLR cascade, oxidative stress-induced senescence, cellular response to hypoxia, and 02-dependent proline hydroxylation of HIF1α.

**Conclusion:** Increased CVD risk in PWH may be driven by trained immunity as evidenced by immune functional profiling of blood-derived MDMs and spatial profiling of macrophages within blood vessel walls. The figure, table, or graphic for this abstract has been removed.

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**II-32 Isoroms Potentially Impact Bone and Cardiovascular Diseases in HIV Infection**

Hardík Ramani1, Madeleine Durand1, Aurelie Ciret-Buhot1, Remi Bunet1, Carl Chartrand-Lefebvre1, Benoit Trassier1, Rejean Thomas1, Jean-Pierre Routy1, Claude Fortin2, Valérie Martel-Laferrière1, Alan Landay3, Mohammad-Ali Jenabian1, Mohamed El-Far4, Cécile Tremblay5

1Centre de Recherche du CHUM, Montreal, Canada, 2Clinique Médicale du Quartier Latin, Montreal, Canada, 3Clinique Médicale (CHT), Montreal, Canada, 4McGill University Health Centre, Montreal, Canada, 5Rush University Medical Center, Chicago, IL, USA, 6Université du Québec à Montréal, Montreal, Canada

**Background:** We have recently demonstrated that the multi-isoform proinflammatory cytokine IL-32 is upregulated by HIV infection and is associated with subclinical CVD in people living with HIV (PLWH). However, mechanisms by which IL-32 isoforms contribute to the pathogenesis of CVD and potentially other chronic diseases like osteoporosis remain unclear. We investigated the impact of IL-32 isoroms α, β and γ on the differentiation of human mesenchymal stem cells (hMSC) and primary human monocytes into osteoblasts or osteoclasts (cells involved in calcium deposition and bone formation or bone resorption, respectively). These cells are involved in processes that are known to be dysregulated in PLWH in the bone (increased osteoporosis) and in the heart (increased non-calcified, vulnerable atherosclerotic plaque). We hypothesize that alterations in IL-32 isoforms seen in PLWH may be a common cause for bone and heart disease seen in that population.

**Methods:** Human CD14+CD16- monocytes or hMSC (osteoblast precursors) were stimulated with recombinant IL-32 isoforms α, β or γ at 500 ng/ml with or without the osteoclast differentiation factor RANKL (30ng/ml) for 21 days. Immunofluorescence imaging on Spinning Disc Zeiss AxioObserver was used to identify and analyze the differentiated cells.

**Results:** Both IL-32α and IL-32β but not IL-32α induced the differentiation of hMSC as well as the primary monocytes into osteocalcin+ osteoblast cells. In contrast, IL-32β but not IL-32α or IL-32β significantly induced the differentiation of a subset of primary CD14+CD16- monocytes into the giant multi-nucleated osteoclasts with the typical expression of the F-actin ring and TRAP+ (p<0.001). Interestingly, when either IL-32β or IL-32γ were combined with RANKL (a typical inducer of osteoclasts), these IL-32 isoforms counteracted the effect of RANKL and maintained their function by inducing osteoblast differentiation from monocytes (p<0.0001 and p=0.0001, respectively), further suggesting their dominant role in osteoblastogenesis.

**Conclusion:** Our data reveal a new function for IL-32 isoforms β and γ (the longest IL-32 isoforms) in inducing the differentiation of primary monocytes into osteocalcin+ osteoblasts by counteracting the osteoclast activation factor RANKL. Meanwhile, IL-32α induces the differentiation of osteoclasts. This novel demonstration of the role of IL-32 in regulation of calcium metabolism suggests that it may constitute a common, upstream cause to patterns of bone and heart disease observed in PLWH.

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**Myocardial Gene Profiling at Time of Sudden Death Demonstrates Increased Immune Responses in PWH**

Tasha Tsao1, David F. Maisson1, Brian H. LaFranchi1, James Salazar1, Ariane Caudal1, Brielle Kinkead1, Kousuke Nakasuka1, Ellen Moffat1, Andrew J. Connolly1, Timothy J. Henrich1, Zian H. Tseng1

1University of California San Francisco, San Francisco, CA, USA, 2Stanford University, Stanford, CA, USA

**Background:** People with HIV (PWH) are at increased risk of sudden cardiac death (SCD). In our HIV Postmortem Systematic Investigation of Sudden Cardiac Death (PMSI-SCD) which is a registry of postmortem samples from PWH, we identified that the majority of PWH who died suddenly had evidence of active infection (92%). A pathophysiologic mechanism underpinning this observation is unknown but may involve active inflammation.

**Methods:** 266 heart samples from sudden cardiac death (SCD) and 256 heart samples from non-HIV-related death (NAD) were collected and subjected to RNASeq. Pathway analysis and protein-protein interaction networks were performed using bioinformatics approaches. Gene ontology and pathway analysis was performed using DAVID and Ingenuity Pathway Analysis.

**Results:** Pathway analysis revealed a significant enrichment of immune-related pathways in PWH relative to NAD samples. The most significant pathways included adaptive immunity, innate immunity, and the TLR signaling pathway. In addition, pathways related to the inflammatory response, stress response, and cell cycle were also enriched in PWH.

**Conclusion:** The findings from this study suggest that increased immune responses and inflammation may play a role in the increased risk of sudden cardiac death in HIV-positive individuals. Further research is needed to understand the mechanisms underlying this association.
Death (POST SCD) Study showed that PWH have higher interstitial myocardial fibrosis, a substrate for fatal cardiac arrhythmias causing SCD. However, the reason(s) for this increase are unknown. The myocardial transcriptome can reflect the acute cellular alterations for these lethal arrhythmias. We leveraged our POST SCD cohort to compare gene expression in myocardium from people with and without HIV infection.

**Methods:** We performed transcriptomic evaluation on left ventricular myocardium sampled at the time of SCD or traumatic death from 245 cases, 20 from PWH, using a Nanostring mCounter panel of 450 curated genes with potential association with SCD. We compared myocardial transcripts from PWH, most of whom were on ART, to 3 uninfected control groups: 1) demographic matched (N=60); 2) cause-of-sudden-death matched (N=20); 3) immediate traumatic deaths (N=11). Gene expression and ontology analyses were performed.

**Results:** We found a significant increase in myocardial expression of CD6 (NK and myeloid immune cell marker) in PWH compared to all 3 uninfected control sudden deaths: demographic, cause-of-death-matched, and immediate traumatic deaths. Moreover, significant increases in myocardial expression of genes related to innate immune response (CD31/PCAM), extracellular matrix homeostasis (MMAP9), DNA damage/apoptosis, and leukocyte-endothelial interactions were observed in PWH compared to uninfected cause-of-death-matched controls. Myocardial CD6 expression was also significantly increased in PWH compared with demographic matched HIV-controls. Statin use did not alter myocardial gene expression in HIV+ SCD. Conversely, collagen-related genes and heart failure markers (ANP/BNP) were downregulated in myocardium from HIV+ SCD compared to that from all 3 HIV- control groups.

**Conclusion:** RNA profiling from myocardium sampled at time of SCD demonstrates increased immune activity but lower expression of genes associated with heart failure in PWH compared to uninfected control sudden deaths. Fibrosis-related genes were also downregulated in HIV+ myocardium, possibly because PWH already had higher levels of pre-existing fibrosis at the time of sudden death. These data suggest that increased risk of SCD in PWH may involve a multi-hit process of increased myocardial immune processes on top of previously established interstitial fibrosis.

**781 Factors Affecting Risk of Major Adverse Cardiovascular Events Among People With HIV in REPRIEVE**

Markella V. Zanni1, Maya Watanabe1, Heather J. Ribaudo1, Gerald S. Bloomfield2, Kathleen V. Fitch1, Carl J. Fichtenbaum1, Trin Umbleja2, Judith S. Currier3, Judith A. Abeg3, Carlos D. Malvestutto4, Marissa Digs1, Michael T. Lu1, Pamela S. Douglas1, Steven K. Grinspoon1, for the REPRIEVE Investigators

1Massachusetts General Hospital, Boston, MA, USA; 2Harvard T.H Chan School of Public Health, Boston, MA, USA; 3Duke University School of Medicine, Durham, NC, USA; 4University of California Los Angeles, Los Angeles, CA; 5Mt Sinai School of Medicine, New York, NY, USA; 6The Ohio State University, Columbus, OH, USA

**Background:** People with HIV (PWH) have a 2-fold increased risk of major adverse cardiovascular events (MACE). REPRIEVE showed pitavastatin reduced MACE by 35% among PWH with low-to-moderate traditional risk. Enhanced understanding of the relative contributions of traditional and HIV-specific risk factors (RF) to MACE may guide concomitant individual- and population-level preventive interventions.

**Methods:** Analyses in the REPRIEVE population were performed for first MACE (including MI, TIA/stroke, revascularization, CV death), with median follow-up of 5.6 years through August 2023. Cox proportional hazards models stratified by randomized treatment group were used to account for treatment differences. Individual models adjusting for each entry RF were fit, and the strongest factors were then combined into a single multivariable adjusted model.

**Results:** Among 7,769 participants, 31.3% were female and 65.2% non-white. Median age was 50 years, LDL 108 mg/dL, 10-year ASCVD risk score 4.5%, diabetes prevalence <1%, and CD4 621 cell/mm3. In unadjusted models, RF associated with a higher (P<0.05) risk of first MACE were: older age, male sex, residence in a high-income region, Black/African American race (within high-income regions), family history of premature CAD, current/former smoking, hypertension (HTN), BMI ≥30 kg/m2, fasting glucose ≥100 mg/dL, lower HDL, eGFR <90 mL/min/1.73 mm2, lower nadir CD4, and detectable HIV-1 RNA (VL). After full adjustment, effects of nadal sex, BMI, glucose, eGFR, and nadir CD4 were no longer apparent (P>0.25). Risk for first MACE was higher for older individuals (50-59 vs ≥60 vs. 40-49, HR's: 1.98 and 2.11), as well as those with a family history of premature CAD (HR: 1.57), Black/African American race (vs. white race, within high-income regions HR: 1.75), current/former smoking (HR: 1.66), HTN (HR: 1.68), and detectable VL (HR: 1.46), and lower for those with higher HDL-C (HR: 0.83). Individuals from a high-income region had a higher risk of first MACE vs. those from other regions, save for those from South Asia.

**Conclusion:** Among a global primary prevention cohort of PWH, factors associated with first MACE after accounting for statin effect included modifiable RF of cigarette smoking, HTN, and detectable VL. A protective effect of female sex was not apparent. Additional work is needed to understand the higher risk of MACE associated with residence in a high-income region, particularly among Black/African American PWH.

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**782 Performance of the ACC/AHA Pooled Cohort Equations for Risk Prediction in the Global REPRIEVE Trial**

Steven K. Grinspoon1, Heather J. Ribaudo2, Virginia Triant3, Kathleen V. Fitch4, Amy Kantor5, Judith S. Currier5, Gerald S. Bloomfield5, Marissa Digs6, Judith A. Abeg7, Carlos D. Malvestutto7, Carl J. Fichtenbaum8, Michael T. Lu9, Markella V. Zanni10, Pamela S. Douglas11, for the REPRIEVE Investigators

1Massachusetts General Hospital, Boston, MA, USA; 2Harvard T.H Chan School of Public Health, Boston, MA, USA; 3University of California Los Angeles, Los Angeles, CA, USA; 4Duke University School of Medicine, Durham, NC, USA; 5Icahn School of Medicine at Mt Sinai, New York, NY, USA; 6The Ohio State University, Columbus, OH, USA; 7University of Cincinnati, Cincinnati, OH, USA

**Background:** People with HIV (PWH) have a higher burden of cardiovascular (CV) disease than the general population; estimating this risk is an essential component of prevention. However, it is unknown how well the 2013 ACC/AHA guideline-recommended Pooled Cohort Equation (PCE) estimates risk among PWH globally. Leveraging the international REPRIEVE Trial, which prospectively adjudicated incident CV events, we compared observed vs. predicted event rates in PWH not taking statins.

**Methods:** The REPRIEVE Trial used the PCE to determine eligibility of PWH at low-moderate CV risk for a statin primary prevention trial. We now assess discrimination and calibration of the PCE in those randomized to placebo (n=3869) as well as those randomized to statin but never starting treatment (n=24). To align with the median 5-year follow up in REPRIEVE, a 5-year risk score was recalculated for this analysis per established method, and follow-up beyond 5 years was censored. We limited outcomes to the specific CV events predicted by the PCE (hard MACE): CV death, myocardial infarction (MI), and stroke. We calculated the C-statistic, the observed: expected (O:E) event ratio and the Harrell's goodness-of-fit (GND) statistic overall and in subgroups by race, natal sex, and Global Burden of Disease region. Small GND p-value indicates poor calibration.

**Results:** Participants were mean age 50 years, 31% female, 65% nonwhite, with a median (Q1, Q3) 10-yr PCE risk of 4.5% (2.2, 7.1). Overall, discrimination was moderate (C-statistic 0.72) and calibration was good (O:E events, 84:91, ratio 1.03, GND P=0.81). However, calibration demonstrated over-prediction of risk outside High Income regions. When restricted to High Income regions, under-prediction (O:E ratio>1.0) was supported among females (2.56) and Black or African American participants (1.66).

**Conclusion:** Among a global cohort of PWH with low-to-moderate traditional CV risk, the PCE was moderately effective to predict CV death, MI or stroke over 5 years but under-predicted events in females, Blacks or African Americans and participants from high-income regions. Performance in selected subgroups should be considered when using the PCE to guide prescribing statin therapy for CV prevention among PWH.

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**783 Residual HIV Viremia Doubles Cardiovascular Disease Incidence Independent of Classic Risk Factors**

Twan Otten1, Marc Blauw1, Wilhelm A. Vo1, Albert L. Groenendijk1, Louise E. van Eekeren1, Olivier Richel1, Mihai Netea1, Jan van Lunzen1, Niels P. Riksen1, Linda Vandekerckhove1, Andre J. van der Ven1, for the HIV Human Functional Genomics Partnership Program

1Radboud University Medical Center, Nijmegen, Netherlands; 2Elisabeth-TweeSteden Ziekenhuis, Tilburg, Netherlands; 3VUmc, Amsterdam, Netherlands; 4Erasmus University Medical Center, Rotterdam, Netherlands; 5Ghent University, Ghent, Belgium

**Background:** People living with HIV (PLHIV) have a heightened risk of atherosclerotic cardiovascular diseases (CVD) beyond that explained by traditional risk factors, potentially driven by HIV-related inflammation or other HIV-related factors. Although antiretroviral therapy (ART) should result in undetectable plasma viral loads (VL), some PLHIV show unquantifiable low yet
decline in CVD incidence need to be investigated further, hypertension may have shown a decline in age-standardised CVD incidence in people with HIV.

Conclusion: The decline in CVD IR.

dyslipidemia) or stage of HIV disease (CD4 nadir, prior AIDS) did not influence CVD risk factors (smoking, chronic kidney disease, body mass index, diabetes, hypercholesterolemia and CVD family history.

Results: At baseline, 665 PLHIV had RV, 3150 had undetectable VL. Participants with RV had higher VL zenith, and shorter ART duration but did not differ in terms of statin or abacavir use, ART adherence and dual DTG based versus three-drug ART nor presence of carotid plaque on ultrasound. Participants with RV more often had a history of myocardial infarction (5.6% vs 2.7% OR 2.28 p=0.01; 71% vs 1.5% OR 9.6 p=0.04 in discovery and validation cohort respectively).

Notably RV participants were twice as likely to develop a first cardiovascular event during follow up (3.9% vs 1.4% OR 2.58 p=0.01). Comparing PLHIV with and without RV, no differences were observed in the functional immunological assays, however plasma levels of TNF Superfamily member 10, Granymes (A, B and H) were higher in participants with RV.

Conclusion: Our data are the first to suggest that residual viremia is a risk factor for past and future cardiovascular diseases independent from traditional CVD risk factors. The mechanism may be through upregulation of specific inflammatory biomarkers that have also been identified for CVD in non-HIV populations.

874 Temporal Trends of Cardiovascular Disease Incidence in People With HIV From 2001-2021

Nadine Jasniski, for the RESPOND and D:A:D Study Groups

Centre of Excellence for Health, Immunity, and Infections, Copenhagen, Denmark

Background: With an increased focus on cardiovascular disease (CVD) as a leading cause of death in people with HIV, monitoring patterns in CVD incidence and its influencing factors in real-life settings is crucial for informed clinical decision-making.

Methods: We followed participants from the D:A:D and RESPOND cohort collaborations from baseline (D:A:D: latest of study entry or 1 Jan 2001; RESPOND: latest of local cohort enrolment or 1 Jan 2012) until the earliest of first CVD event (myocardial infarction (MI), stroke, invasive cardiovascular procedure (ICP)), final follow-up, or 1 Feb 2016 (D:A:D)/31 Dec 2021 (RESPOND). We calculated age-standardised CVD incidence rates (IRs) two-yearly from 2001–2021 and assessed temporal trends by Poisson regression, adjusting for time-updated potential confounders and cohort.

Results: Of 66,680 included individuals, 18% were age >50 (median 40, interquartile range IQR 33–47) at baseline, 74% were male, 38% current smokers, 45% had dyslipidemia, 8% hypertension, 3% diabetes, and 1% prior CVD. Median CD4 cell count was 437 (270–630). Over a median of 8.8 (4.5–13.1) years (586,510 person-years, PY), there were 2,811 CVD events (IR 4.79/1000 PY [95% confidence interval (CI) 4.62–4.97]; 1363 MIs, 768 strokes, 680 ICPs). While the crude CVD incidence remained relatively stable over time, age-standardised IRs decreased from 8.65/1000 PY in 2001–2002 to 3.74/1000 PY in 2019–2021, IR ratio (IRR) 0.30 (95% CI 0.24–0.38, p<0.0001), with a steeper decline up to 2009 (Fig. A). The prevalence of most CVD risk factors was similar or decreased due to increased monitoring. Adjusting for hypertension accentuated the temporal CVD trends (IRR 0.26 [95% CI 0.20–0.32], p<0.0001) while changes in demographics (gender, ethnicity, mode of HIV acquisition), other known CVD risk factors (smoking, chronic kidney disease, body mass index, diabetes, dyslipidemia) or stage of HIV disease (CD4 nadir, prior AIDS) did not influence the decline in CVD IR.

Conclusion: Combining data from two large, international collaborations, we have shown a decline in age-standardised CVD incidence in people with HIV from 2001 to 2021, most pronounced from 2001 to 2009. While causes of the decline in CVD incidence need to be investigated further, hypertension may have contributed to a slower decline over time. The CVD decline did not appear to be affected by changes in demographics, HIV stage and most known CVD risk factors.
without HIV. We assessed factors associated with smoking cessation among cigarette smokers seen in care at outpatient clinics of the HIV Outpatient Study (HOPS).

**Methods:** We analyzed HOPS participants’ data from clinic electronic medical records (EMR) and from an optional annual participant survey from January 1, 2007 to December 31, 2022. We included PWH with EMR or survey evidence of current cigarette use and no smoking cessation medication use at baseline (i.e., first HIV visit) and those who began smoking cigarettes during follow-up. We identified smoking-related comorbidities based on lab results, clinical diagnoses, and treatments. Smoking quit attempts were determined via EMR and survey data during follow-up. We studied associations of first quit attempt with comorbidity diagnoses, smoking cessation medications prescriptions, sociodemographics, and clinical factors using the counting process within Cox proportional hazards analyses.

**Results:** Among 1,068 eligible PWH, 77% were men, 32% White persons, 48% Black persons, 18% Hispanic/Latino persons, 47% aged 40 and older. At baseline, 172 (16%) had chronic obstructive pulmonary disease (COPD)/emphysema, cardiovascular disease (CVD), and/or cancers. During a median follow-up time of 4.4 years (interquartile range: 1.9-6.6), 301 (28%) PWH were prescribed smoking cessation medications (varenicline=87, bupropion=50, nicotine products=323), and 198 (19%) quit smoking. Of these 198, 33 (17%) resumed smoking after first quit. In multivariable analysis, factors positively (p<0.05) associated with smoking cessation included: later year of HOPS enrollment, non-Hispanic/Latino (NH) white race/ethnicity versus NH Black, heterosexual HIV transmission versus men who have sex with men risk group, having a depression diagnosis, being prescribed varenicline, bupropion or nicotine products, whereas factors inversely associated included: being uninsured versus having private insurance, and having diagnoses of psychosis, hypertension, or obesity (Figure).

**Conclusion:** Fewer than one-third of HOPS participants who smoked cigarettes had a prescription for smoking cessation medication, and only one-in-five had a documented quit attempt, highlighting challenges for and need to strengthen prevention of smoking-related chronic diseases in PWH.

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**878 Modifiable Factors Associated With Cardiovascular Disease Risk Among Women With and Without HIV**

Jenni Wise, Elizabeth Jackson, Liang Shan, Andrew Edmonds, Deborah Konkle-Parker, Maria L. Alcadel, Gina Wiipood, Tracey Wilson, Kathleen Weber, Aruna Chandra, Seble Kassaye, David B. Hanna, Anna Leddy, John Cleveland, Mirjam Colette-Kempf, for the Multicenter AIDS Cohort Study (MACS) and Women's Intergenerational HIV Study (WHIS) Combined Cohort Study

1. University of Alabama at Birmingham, Birmingham, AL, USA,
2. University of North Carolina at Chapel Hill, Chapel Hill, NC, USA,
3. University of Mississippi Medical Center, Jackson, MS, USA,
4. University of Miami, Miami, FL, USA,
5. Emory University, Atlanta, GA, USA,
6. State University of New York Downstate Medical Center, Brooklyn, NY, USA,
7. Cook County Health & Hospitals System, Chicago, IL, USA,
8. The Johns Hopkins University, Baltimore, MD, USA,
9. Georgetown University, Washington, DC, USA,
10. Albert Einstein College of Medicine, Bronx, NY, USA,
11. University of California San Francisco, San Francisco, CA, USA

**Background:** Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among women in the United States. Women living with HIV (WWH) have twice the risk of CVD events compared to women without HIV (WWoH). While increased CVD risk for WWH has been largely attributed to HIV-specific factors, interventions on modifiable factors (e.g., social support, psychological health, physical activity, diet quality, substance use, and HIV viral suppression) may decrease CVD risk among women.

**Methods:** We analyzed data from the Women’s Intergenerational HIV Study (WHIS) to examine relationships between individual-level factors and CVD risk among WWH and WWoH. Women were included if they were 30-79 years of age at the time of their semiannual WHIS study visit (April – September 2019) and had data to calculate the American College of Cardiology and American Heart Association Pooled Cohort Risk Equation (PCE), a 10-year CVD risk score. Descriptive statistics and logistic regression were used to test associations between each factor and CVD risk among WWH and WWoH. CVD risk was dichotomized as low risk versus high risk, and four-year and ten-year outcomes were examined. Differences in odds ratios were calculated across categorical variables and study populations.

**Results:** Data was available for 1,711 women (72% HIV+) with a median age of 53 years (IQR: 46-58). Approximately half (53%) were classified as having low (<5%) risk, while 13%, 24%, and 9% were classified as having borderline (5-7.4%), moderate (7.5-19.9%), or high risk (≥20%), respectively. 45.7% of WWH and 51.6% of WWoH were classified as having borderline or higher risk for CVD. Higher annual household income (OR: 0.57, 95% confidence interval [CI]: 0.45-0.72), greater physical activity (OR: 0.63, 95% CI: 0.54-0.73), better diet quality (OR: 1.03, 95% CI: 1.00-1.06), and <7 alcoholic drinks per week (OR: 0.63, 95%
CI: 0.51-0.77) were associated with decreased odds of higher CVD risk, while current smoking (OR: 2.22, 95% CI: 1.81-2.70) was associated with increased odds of higher CVD risk. We found effect modification of smoking by HIV status, with an OR for higher risk of 2.54 (95% CI: 2.00-3.21) among WWH versus 1.53 (95% CI:1.07-2.21) among WWHo. There was a lack of modification by HIV-status for all other variables tested.

Conclusion: Interventions targeting modifiable lifestyle factors should be considered as a means to reduce CVD risk and improve outcomes among WWH and women behaviorally at risk for acquiring HIV.

**No Increased Risk for Hypertension With CAB-LA Compared to TDF/FTC for HIV PrEP in HPTN 083**

Raphael J. Landovitz1, Heather I. Ribaudo2, Youngjung Choi3, Kari Chansky2, Zoe Wang2, Mina Hosseinipour2, Sinead Delany-Morettie4, Lydia Soto-Torres5, Sheryl Zwerski6, Marybeth McCauley7, James F. Rooney8, Alex R. Rinehart9, Beatriz Grinszte10

1University of California Los Angeles, Los Angeles, CA, USA, 2Harvard T.H Chan School of Public Health, Boston, MA, USA, 3Fred Hutchinson Cancer Center, Seattle, WA, USA, 4University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 5University of the Witwatersrand, Johannesburg, South Africa, 6National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA, 7FHI 100, 8Washington, DC, USA, 9Gilead Sciences, Inc, Foster City, CA, USA, 10HIV Healthcare, Research Triangle Park, NC, USA, 11Oswaldo Cruz Foundation – Fiocruz, Rio de Janeiro, Brazil

**Background:** Results from some trials of HIV treatment suggest an excess risk of hypertension (HTN) associated with integrase strand transfer inhibitors (INSTIs), independent of changes in weight or BMI. HPTN 083, in HIV-negative MSM and transgender women, compared injectable cabotegravir (CAB) to daily oral tenofovir disoproxil fumarate–emtricitabine (TDF–FTC) for HIV PrEP. We performed a post-hoc analysis of HPTN 083 to compare incidence rates for HTN in the absence of HIV infection.

**Methods:** Of 4566 participants (pts) enrolled, the analysis population included 3971 (1993 CAB/1978 TDF–FTC) exposed to study drug and without pre-existing HTN. Incident hypertension was defined as a new diagnosis of HTN, 2 sequential measures of SBP ≥ 140 mm Hg, DBP ≥ 90 mm Hg, or initiation of anti-HTN treatment, through 161 weeks of follow-up. Changes in blood pressure over time by study arm were estimated with mixed effect models. Cox regression models were used to estimate the hazard ratio (HR) for incident HTN between study arms, unadjusted and adjusted for race, age, baseline (BL) BMI, anti-HTN treatment, and time-updated weight change from BL (TUWC).

**Results:** Pts were well matched between the arms (75% were under age 30; 4% were over age 45. 58% were normal or overweight at baseline. Over 10293 person-years of follow-up, median BMI increased +1.2 (IQR 0.2-3.2) kg/m² for CAB and 0.8 (IQR -0.2-2.1) for TDF–FTC. BMI and SBP were weakly correlated. 461 (12%) pts had incident HTN (246 CAB, 215 TDF–FTC). Mean change per year in SBP was 0.56 mm Hg (95% CI 0.41, 0.71) and DBP was 0.73 (0.62, 0.85) in CAB and 0.30 (0.15, 0.45) and 0.66 (0.55, 0.78) in TDF–FTC. Cumulative incidence of HTN over 3 years was 13.4% for CAB and 11.8% for TDF–FTC (Figure). HTN was more common in US and African pts and those with higher BMI, older age, and taking anti-HTN meds for non-HTN indications (MFnH). Between-arm differences were numerically larger for US pts, older pts, and pts taking MFnH. HR for incident HTN was 1.11 (95% CI 1.05, 1.18, p=0.001) for CAB vs. TDF–FTC in the unadjusted analysis, and 1.00 (95% CI 0.83, 1.21) after adjustment for race, BL age, BL BMI, anti-HTN BL meds, and TUWC.

**Conclusion:** HTN incidence in HPTN 083 was low. Observed HTN incidence was higher in CAB vs. TDF–FTC, but was not statistically significant. Risk factors for HTN included age, BL, obesity, and weight gain over time. Over 3 years there was no increase in the hazard of incident HTN in CAB vs. TDF–FTC after adjusting for relevant covariates.

**Hypertension Treatment Gap Among People With/Without HIV in Kenya, Nigeria, Tanzania, and Uganda**

Matthew Romeo1, Nicole Dear1, Trevor A. Crowell2, Seth Frndak1, Hannah Kibukwa1, John Owoothu2, Valentine Singo3, Jonash Maswai1, Emmanuel Bahamena1, Victor Anyebe1, Zahra Parker1, Julie Aké1, J. Sean Cavanaugh2, Neha Shai3, for the African Cohort Study (AFRICOS) Group


**Background:** Mortality associated with hypertension (HTN) in sub-Saharan Africa is among the highest globally and unlike other regions, little progress has been made in diagnosis, treatment, and control. HIV care programs have typically focused on providing HIV-related care, but as life expectancy of people with HIV (PWH) are extending, addressing noncommunicable diseases and risk factors may be increasingly important.

**Methods:** The prospective African Cohort Study enrollment PWH and people without HIV (PWWH), aged ≥15 years, in care at 12 PEPFAR-supported facilities in Kenya, Nigeria, Tanzania, and Uganda. Among participants with at least two 6-monthly study visits, we defined HTN as a persistently elevated systolic and/or diastolic blood pressure (BP) ≥140/90 mmHg at ≥2 consecutive visits, or receipt

**Higher Risks of Hypertension With Use of DTG Versus PI/r in the VISEND Trial**

Lloyd B. Mulenga1, Kaitlyn M. McCann1, Lamiek Chirwa2, Manya Mirchandani1, Andrew Hill3

1University Teaching Hospital, Lusaka, Zambia, 2Imperial College London, London, United Kingdom, 3University of Liverpool, Liverpool, United Kingdom

**Background:** Hypertension is a leading cause of death in sub-Saharan Africa. In the general population, risks of hypertension rise with increasing age and body weight. Many African HIV treatment programs do not include funding for treatment of hypertension. Use of tenofovir alafenamide (TAF) and dolutegravir (DTG) have been associated with higher risks of hypertension in some randomized trials and cohort studies, but other studies have not shown these associations. The VISEND study was conducted in Zambia to evaluate the safety and efficacy of second-line treatment, after NNRTI failure.

**Methods:** The VISEND study recruited adults previously taking NNRTI based treatment. Participants with HIV-1 RNA <1,000 c/mL at screening (Low VL Group) were randomized to TDF/3TC/DTG or TAF/FTC/DTG. Participants with HIV-1 RNA >1,000 c/mL (High VL Group) were randomized to TDF/3TC/DTG, TAF/FTC/DTG, ZDV/3TC/LVP/r or ZDV/3TC/ATV/r. Blood pressure was evaluated at study Weeks 24, 48, 96 and 144. The rate of 1% incidence (SBP/DBP >140/90 mmHg) was compared between the TDF/3TC/DTG and TAF/FTC/DTG arms, by use of DTG vs PI/r, using Cochran Mantel-Haenszel tests.

**Results:** At Week 24, there were no significant differences in Grade 1 HTN between treatment arms within either VL Group. Across the VL Groups, rises in BMI were significantly higher for people taking TAF/FTC/DTG versus TDF/3TC/DTG (p<0.001). In the high VL Group, rises in BMI were significantly greater in the TAF/FTC/DTG or TDF/3TC/DTG arms, versus the ZDV/3TC/LVP/r or ZDV/3TC/ATV/r arms (p<0.001). Systolic BP increased across TAF/FTC/DTG, TDF/3TC/DTG, and ZDV/3TC/LVP/r arms, while no changes were noted in the ZDV/3TC/ATV/r arm. The prevalence of hypertension increased over time in all treatment arms. By Week 144, the percentage with Grade 1 hypertension was significantly higher for people taking TAF/FTC/DTG, compared with TDF/3TC/DTG (p=0.02, stratified by VL Group). By Week 144 there were significantly more people in the TDF/3TC/DTG and TAF/FTC/DTG arms with Grade 1 HTN (173/343, 50%) compared with the ZDV/3TC/LVP/r or ZDV/3TC/ATV/r arms (76/258, 27%) (p<0.001).

**Conclusion:** In VISEND, risks of Grade 1 HTN and changes in systolic BP were higher for TAF/FTC/DTG and TDF/3TC/DTG versus ZDV/3TC/ATV/r or ZDV/3TC/LVP/r. Results were not consistent when comparing TAF/3TC/DTG and TDF/3TC/DTG, where there were no significant differences for change in systolic BP, but there were differences when comparing the risk of Grade 1 HTN.
of any HTN medication. All subsequent study visits were classified as having HTN. Multivariable random intercept log-Poisson models with robust standard errors that included HIV status, time in the cohort, demographic and clinical characteristics, and site service delivery characteristics were used to examine associations with study visit-level rates of untreated HTN and uncontrolled BP (≥130/80 mmHg) among those on HTN treatment.

Results: From 1/2013–6/2023, 4114 participants were enrolled; 3638 with a total of 19,507 person-years of follow-up were included. Overall, 693 (19%) ever had HTN, among whom median (IQR) age was 48 (41–54) years, 591 (85%) were PWH, and 380 (55%) were female. Of those with HTN, 414 (60%) never received HTN treatment (figure). Among those on HTN treatment, 87% had one or more study visits with uncontrolled BP, with a median (IQR) proportion of study visits with uncontrolled BP of 73% (43%–100%). At their most recent study visit, 158 (57%) of those on HTN treatment received combination treatment as recommended by WHO guidelines. In multivariable models, HIV status was not significantly associated with untreated HTN (PWH vs. PWoH: adjusted rate ratio [aRR] 0.93, 95% CI: 0.84–1.03) or uncontrolled BP (PWH vs. PWoH: aRR 0.97, 95% CI: 0.81–1.17). Males had a significantly higher rate of untreated HTN (aRR 1.18, 95% CI: 1.08–1.28), but not uncontrolled BP (aRR 1.10, 95% CI: 0.96–1.26).

Conclusion: We identified a substantial burden of untreated and uncontrolled HTN, unaffected by HIV status. Strategies are needed to optimally scale up HTN diagnosis and management in the context of existing HIV treatment services for PWH and testing/prevention services for PWoH.

HIV-Associated Heart Failure: Phenotypes and Clinical Outcomes in a Safety Net Setting
Matthew S. Durstenfeld, Anjali Thakkar, Yifei Ma, Priscilla Y. Hsue
University of California San Francisco, San Francisco, CA, USA

Background: HIV is associated with increased risk of heart failure and with worse outcomes after diagnosis with heart failure. However, whether cardiomyopathy phenotypes vary by HIV status and whether HIV is associated with similarly increased risk within a contemporary population who receives care within a safety-net system is unknown.

Methods: In this observational study, using an electronic health record database of all individuals with diagnosed heart failure within the San Francisco Health Network, a municipal safety-net system, from 2001-2019, we compared individuals with and without HIV (defined by ICD code and at least one CD4 count or viral load) in terms of baseline characteristics, heart failure phenotypes by ejection fraction and presumed etiology, and outcomes after heart failure diagnosis including all-cause mortality (linked to national death statistics), and hospitalization for heart failure.

Results: We included 14,829 individuals with HF, of whom 697 (4.7%) had HIV. Although 47% without HIV were female, only 19.7% with HIV were female with HIV, and the median age was 63 without HIV and 53 with HIV at time of HF diagnosis. Among those with HIV, the median nadir CD4 count was 133 (IQR 44, 275), and at HF diagnosis was 356 (IQR 173, 566). Persons with HIV had much higher proportions with documented alcohol, tobacco, opioid, cocaine and methamphetamine use (p<0.001 for each). 38% vs. 33% had a reduced ejection fraction to <40% (p=0.009). There were no differences in the proportion with prior myocardial infarction or obstructive coronary artery disease on angiography. Median survival was 6.1 years (95% CI 5.4–7.1) among people with HIV compared to 11.3 years without HIV (95%CI 10.7–11.7). HIV was associated with higher risk of all-cause mortality (HR 1.55 95%CI 1.38–1.74; p<0.001; Figure) and lower odds of heart failure hospitalization (OR 0.51; 95%CI 0.39–0.66; p<0.001). Lower nadir CD4 and CD4 at the time of HF diagnosis were associated with worse survival: HR 1.37 for nadir < 200 (95%CI 1.08–1.73; p=0.009) and HR 1.69 for current <200 (95%CI 1.34–2.13; p<0.001), respectively. Viral load greater than 500 copies/ml was not associated with mortality (HR 1.02; 95%CI 0.80–1.31; p=0.87).

Conclusion: Among people with HF who receive care within a municipal safety-net system, HIV infection is associated with diagnosis with HF ten years earlier and significantly elevated risk of mortality despite lower risk of HF hospitalization.

Repeated Stimulant Use in the Context of Left Ventricular Dysfunction among Women Living with HIV
Elise D. Riley, Yifei Ma, Sampy Shibole, Katherine C. Wui, Torsten Neillandi, Adam W. Carrico, Claudia Martines, Denise Vidor, Carlos Rodriguez, Aruna Chandran, Kathleen Weber, Jason Lazaro, Antonina Foster, Phyllis Tien, Jorge R. Kizer
1University of California San Francisco, San Francisco, CA, USA, 2The Johns Hopkins Hospital, Baltimore, MD, USA, 3Florida International University, Miami, FL, USA, 4University of Miami, Miami, FL, USA, 5Albert Einstein College of Medicine, Bronx, NY, USA, 6The Johns Hopkins University, Baltimore, MD, USA, 7Yeshen Institute of Medicine, Chicago, IL, USA, 8State University of New York Downstate Medical Center Downstate Medical Center, Brooklyn, NY, USA, 9Emory University, Atlanta, GA, USA

Background: HIV is associated with an increased risk of heart failure (HF), which is especially pronounced in women. This is concerning because women with HIV (WWH) come predominantly from vulnerable race-ethnic minority groups that are at high risk for HF. In addition, people with HIV have a disproportionately high prevalence of substance use, which is also associated with heart disease. The extent to which specific substances or substance types relate to precursors of clinical HF, such as left ventricular systolic dysfunction (LVDD) and diastolic dysfunction (LVDD), in WWH remains under-studied.

Methods: We investigated cross-sectional associations between repeated use of various substances and pre-HF phenotypes, LVDD and LVDD, in the Women’s Interagency HIV Study. We defined exposures as self-reported substance use (i.e., tobacco, alcohol, cannabis, cocaine, methamphetamine/amphetamine, opioids, and sedatives) at ≥2 study visits (“repeated use”) compared to no use or single-visit use. Standardized echocardiograms were performed from 2014 to 2019 in 1,162 WWH. LVDD and LVDD were defined by American Society of Echocardiography criteria.

Results: Most participants (75%) identified as non-Hispanic Black, the average age was 49 years, 58% were post-menopausal, the median nadir CD4 cell count over the study period was 478, and 55% of participants were virally suppressed at all study visits. Repeated substance use ranged from 1.3% (cocaine use) to 66.1% (alcohol). Among all participants, 5.5% had LVDD. None of the repeated substance use measures were significantly associated with LVDD. Among participants without LVDD, 6.1% had LVDD. Adjusting for socioeconomic and clinical risk factors, the odds of LVDD were 5.22 times higher among women who reported repeated methamphetamine/amphetamine use (95% CI: 1.10, 24.73) and 1.87 times higher among those who reported repeated use of tobacco (95% CI: 1.10, 3.17). HIV-specific factors and other substances did not reach levels of significance.

Conclusion: Repeated tobacco use and, to a much larger extent, repeated methamphetamine/amphetamine use had strong associations with LVDD in this population. Our findings highlight stimulant use as a potent risk factor for LVDD among WWH. Assessment and targeted interventions for WWH who use methamphetamine/amphetamine may lower cardiovascular risk in this population.
794 Proteomic Signature of HIV-Associated Myocardial Fibrosis and Incident Heart Failure
The Johns Hopkins University School of Medicine, Baltimore, MD, USA; National Institutes of Health, Bethesda, MD, USA; The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
Background: Factors linked to reduced myocardial flow reserve in HIV using 82Rb proteomic signature of HIV-associated myocardial fibrosis and incident had proximal blood tests taken within 6 months before and 3 months after the and CD8+ T-cell counts were collected from electronic records; 69/72 (95%) normal (>2.0). Plasma HIV-1 RNA levels, nadir CD4+ T-cell and proximal CD4+ disease (CKD, p=0.024) and CAD (p=0.025), but not with age, sex, body mass index, HIV suppression, hypertension, diabetes mellitus, hyperlipidemia, congestive heart disease, stroke or peripheral vascular disease. PWH with low MFR had lower CD4+ T-cell (median 364 [IQR 333,634] vs. 621 [IQR 429,866], p=0.040) and nadir CD4+ T-cell counts (median 176 [IQR 74,285] vs. 296 [IQR 192,432], p=0.017) when compared to PWH with normal MFR.
Conclusion: In addition to CD4 and CAD, PWH who have lower nadir current CD4+ T-cell counts, indicating a more compromised immune function, may be at a higher risk of coronary microvascular dysfunction.

795 Diastolic Dysfunction With Preserved Ejection Fraction in Humanized HIV-Female Mice on cART
Keshore R. Bidasee, Prasanta Dash, Fadhel A. Alomar, Zachary L. Venn, Chen Zhang, Rongyu Tu, Lili Guo, Bryan T. Hackfort, Santhi Gorantla University of Nebraska Medical Center, Omaha, NE, USA
Background: Early-onset diastolic dysfunction has emerged as a major threat to healthy aging in people with HIV-1 infection. Women living with HIV-1 infection (WLWH) are especially vulnerable and develop a unique pattern vascular and myocardial ischemia compared to men. Animal models that recapitulate this pathophysiology remain unreported, and this has left a void in our understanding of the molecular causes of the disease and treatment strategies to alleviate it.
Methods: Female NOD.Cg-PtkdcscidIl2rgtm1Wjl/Sj2l humanized mice (Hu-mice) were infected with HIV-1ADA and treated for fourteen weeks with dolutegravir (DTG)/tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) starting two weeks of infection. In vivo echocardiography was used to assess cardiac function and dimensions. Photoacoustic imaging was used to assess saturated oxygenated hemoglobin in the anterior wall of the heart. Prior to sacrifice, animals were intravenously injected with the fluorescent dye cardiac function and dimensions. Photoacoustic imaging was used to assess cardiac function and dimensions. Photoacoustic imaging was used to assess saturated oxygenated hemoglobin in the anterior wall of the heart. Prior to sacrifice, animals were intravenously injected with the fluorescent dye Dylight 705. Results: Participants included 45 (61%) males, with a median age of 59 [IQR 53,64]; 67 (91%) were virally suppressed (<50 cps/ml). Their CD4+ and CD8+ T-cell counts were 572 [IQR 371,820] and 713 [IQR 438,981] cells/mm³, with a CD4/CD8 ratio of 0.91 [IQR 0.67,1.21]. Their nadir CD4+ T-cell count was 282 [IQR 133,361] cells/mm³. The resting and stress MBF were 0.94 [IQR 0.71,1.16] and 2.11 [IQR 1.65,2.42] ml/min/g, respectively, while the MFR was 2.21 [IQR 1.84,2.63]. Forty-five (61%) had a normal global MFR, while 9 (12%) had a low MFR. In the entire group, a lower MFR was associated with chronic kidney disease (CKD, p=0.024) and CAD (p=0.025), but not with age, sex, body mass index, HIV suppression, hypertension, diabetes mellitus, hyperlipidemia, congestive heart disease, stroke or peripheral vascular disease. PWH with low MFR had lower CD4+ T-cell (median 364 [IQR 333,634] vs. 621 [IQR 429,866], p=0.040) and nadir CD4+ T-cell counts (median 176 [IQR 74,285] vs. 296 [IQR 192,432], p=0.017) when compared to PWH with normal MFR.
Conclusion: In addition to CD4 and CAD, PWH who have lower nadir current CD4+ T-cell counts, indicating a more compromised immune function, may be at a higher risk of coronary microvascular dysfunction.

796 Factors Linked to Reduced Myocardial Flow Reserve in HIV Using 82Rb Positron Emission Tomography
Samantha Sithole, Serena Spudich, Mehran Sadeghi, Edward J. Miller, Attila Feher, Phillip Chan
Yale University, New Haven, CT, USA
Background: Despite stable HIV suppression and substantial immune reconstitution through antiretroviral therapy, people with HIV (PWH) demonstrate an elevated risk of coronary artery disease (CAD) and elevated levels of endothelial injury markers. Myocardial flow reserve (MFR) is an indicator of coronary microvascular dysfunction and overall vascular health. This retrospective study sought to evaluate the association between HIV-related parameters and MFR, determined by the stress over rest myocardial blood flow (MBF) using 82Rb/201Tl positron emission tomography (PET) and PWH in Baltimore/DC and Chicago (SMASH). Myocardial fibrosis was measured by extracellular volume fraction (ECV). Clinical findings were assessed in individuals and as clusters defined using weighted gene co-expression network analysis. We estimated associations with HIV and elevated ECV (≥30% in women, ≥28% in men) using multivariable regression and explored protein relationships using annotated enrichment analysis. We tested associations of identified signatures with incident adjudicated HF using Cox regression analysis among older PWH (MESA).
Results: Among 342 SMASH participants (age 55±6 years), 52% had elevated ECV and 61% were PLWH (88% on ART, 74% with undetectable plasma HIV RNA). Of 2594 proteins, 439 were associated with HIV seropositivity (SP) (p<14 excluding PLWH with detectable plasma HIV RNA) with a false discovery rate <0.05. We identified 39 (32) of these proteins as candidate contributors to the independent association between HIV SP and elevated ECV, including 9 proteins involved in apoptosis, 8 in cytokine signaling, 6 in T-cell activation, and 6 in the MAPK cascade. These proteins cluster associated with elevated ECV and HIV SP regardless of HIV viral suppression status, enriched in factors involved in TNF signaling, ephrin signaling, and extracellular matrix (ECM) organization. This protein cluster as well as 31 of 39 individual proteins were associated with incident HF among 2273 PWH in MESA (age 68±9 years; 8.5±1.4 years of follow-up).
Conclusion: Proteomic signatures that may in part reflect or contribute to HIV-associated myocardial fibrosis are enriched in pathways of immune activation, cytokine signaling, and ECM organization, even among virally suppressed PLWH. These signatures also predict incident HF in a large independent cohort of older PWH, suggesting underlying pathways that may portend risk of HF among PLWH may also drive HF pathogenesis among PWH. The figure, table, or graph for this abstract has been omitted.
797 Impact of Semaglutide on Weight Change Among People With HIV: A Stratified Analysis by Baseline BMI
Lara Haidar1, Heidi M. Crane2, Robin M. Nance3, Allison R. Wеб4, Geetaanjali Chander5, Bridget Whitney6, Amanda Willig7, Lynsyde S. Mixson8, Alekhyा Lavu9, Laila Aboulatta10, Mindy Dai2, Andrew Hahn11, Edward Cachay12, Lydia N. Drumright13, Sherif Etteny14
1University of Manitoba, Winnipeg, Canada, 2University of Washington, Seattle, WA, USA, 3University of Alabama at Birmingham, Birmingham, AL, USA, 4University of California San Diego, San Diego, CA, USA
Background: Limited real-world evidence on effectiveness of semaglutide for weight loss among people with HIV (PWH) exists. We aimed to investigate weight change in a cohort of PWH initiating semaglutide use.
Methods: Adult PWH who initiated semaglutide between 2018 and 2022 and had >2 weight measurements from the Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) cohort were assessed for within-person (1) bodyweight change in kg at 1 year and (2) percent bodyweight change using linear mixed model adjusted for age, sex, race/ethnicity, CNICS site, diabetes status, CD4 cell count, HIV viral load (VL), and time. We also investigated whether the effect of semaglutide on weight change varied by baseline BMI category, diabetes status, and semaglutide dose.
Results: During the study period 222 PWH initiated semaglutide. Mean follow up was 1.1 years. Approximately 75% were male. At baseline mean age was 53 years (standard deviation [SD]: 10), average weight was 108 kg (SD: 23), mean BMI was 35.5 kg/m², mean HbA1c was 7.7% and 77% had clinically recognized diabetes. At baseline, 97% were on ART and 89% were virally suppressed (VL<50 copies/mL). The majority, 87 (69.6%) received low doses of subcutaneously injected semaglutide (0.25, 0.5, and 1 mg), while 24 (19.2%) received high doses of subcutaneously injected semaglutide (1.2, 2.4, mg). In linear mixed models, treatment with semaglutide was associated with an average weight loss of 6.5 kg at 1 year (95% CI -7.7, -5.2) and a percent bodyweight reduction of 5.7% (-6.9 to -4.6) at 1 year. Reductions in weight among PWH were -4.1 (-7.9, -0.2) kg (p < 0.04) with normal BMI, -4.6 (-6.9, -2.3) kg (p < 0.01) in overweight, -5.4 (-7.3, -3.4) kg (p < 0.01) in obesity class 1, -7.6 (-9.5, -5.7) kg (p < 0.01) in obesity class 2, and -8.8 (-10.9, -6.7) kg (p < 0.01) in obesity class 3. There was also a significant difference in weight loss between PWH with obesity class 3 (reference) and PWH with normal BMI, overweight, and obesity class 1 (p for interaction <0.05). No significant differences in weight loss by diabetes status or semaglutide dose were observed.
Conclusion: Among PWH, semaglutide was associated with significant weight loss, with more substantial weight loss observed in individuals with higher BMI. These findings are highly relevant given high proportions of diabetes, overweight, and obesity among PWH.

798 Effects of Semaglutide on Inflammation and Immune Activation in HIV-Associated Lipohypertrophy
Allison Ross Eckard1, Qian Wu2, Abdu Satta3, Nicholas Funderburg4, Danielle Labbate1, Grace L. Ditzelbernger5, Jordan E. Lake6, Douglas W. Kitch7, Amy Kantor1, Raja Muthupillai1, Pablo Belaunzaran-Zamudio2, Todd T. Brown8, Kathleen Corey9, Alan Landay10, Anchalee Avihingsanon11, Fred R. Sattar12, Kristine M. Eralson13
1University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 2University of Texas at Houston, Houston, TX, USA, 3Harvard T H Chan School of Public Health, Boston, MA, USA, 4Baylor College of Medicine, Houston, TX, USA, 5National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA, 6Johns Hopkins University School of Medicine, Baltimore, MD, USA, 7Massachusetts General Hospital, Boston, MA, USA, 8University of North Carolina School of Medicine, Chapel Hill, NC, USA, 9University of Queensland, Brisbane, Australia, 10Rush University, Chicago, IL, USA, 11Thailand Red Cross AIDS Research Center, Bangkok, Thailand, 12University of Southern California, Los Angeles, CA, USA
Background: Semaglutide, a GLP-1 receptor agonist, is a highly effective medication for decreasing weight and weight complications by suppressing appetite, improving insulin signaling, and reducing intrahepatic triglycerides (IHTG). However, concomitant loss of muscle mass often accompanies weight loss, which may have consequences on muscle function. The purpose of this analysis was to examine changes in muscle mass, quantity, and function among people with HIV (PWH) treated with semaglutide for metabolic-associated steatotic liver disease (MASLD).
Methods: We leveraged data from the SLIM LIVER (ACTG AS371) study, a single-arm pilot of the effects of semaglutide on IHTG in PWH with MASLD. Participants received subcutaneous semaglutide for 24 weeks (titrated to 1 mg/week by week 4). Poosas volume/fat fraction were assessed from liver magnetic resonance imaging and physical function by 10-time chair rise test and 4m gait speed, at baseline and week 24. Test changes were observed for soluble interleukin-6 (sIL-6) (2.51 [2.05, 2.13] (1.85) pg/mL; P=0.016), high-sensitivity C-reactive protein (hsCRP) (2.98 [2.69, 1.83] (2.96) pg/mL; P=0.008) (Figure 1), and sCD163 (511 [1.54] ng/mL; P=0.005) with a trend in sCD14 (1694 [1.3], 1575 [1.28] mg/mL; P=0.085). No significant changes were observed for soluble interleukin adhesion molecule-1 (sICAM-1) or TNF-receptor/1-2. Biomarker levels did not change significantly within the placebo group. Treatment effects of semaglutide were significant in regression analyses (β coefficient [95% confidence interval]) for log IL-6 (-0.30 [-0.54, -0.06]; P=0.015) and log hsCRP (-0.51 [-0.90, -0.12]; P=0.011) with trends in log sCD14 (-0.08 [-0.18, 0.01]; P=0.083) and log sICAM-1 (-0.11 [-0.24, 0.02]; P=0.088).
Conclusion: In non-diabetic PWH with lipohypertrophy, semaglutide had significant effects on several key biomarkers associated with CVD in HIV. Further investigation is warranted to determine the effect on co-morbidities in HIV.

799 Effects of Semaglutide on Muscle Structure and Function in the SLIM Liver Study
Grace L. Ditzelbernger1, Jordan E. Lake2, Douglas W. Kitch3, Amy Kantor4, Raja Muthupillai5, Pablo Belaunzaran-Zamudio6, Todd T. Brown7, Kathleen Corey8, Alan Landay9, Anchalee Avihingsanon10, Fred R. Sattar11, Kristine M. Eralson12
1University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 2University of Texas at Houston, Houston, TX, USA, 3Harvard T H Chan School of Public Health, Boston, MA, USA, 4Baylor College of Medicine, Houston, TX, USA, 5National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA, 6Johns Hopkins University School of Medicine, Baltimore, MD, USA, 7Massachusetts General Hospital, Boston, MA, USA, 8Rush University, Chicago, IL, USA, 9Thailand Red Cross AIDS Research Center, Bangkok, Thailand, 10University of Southern California, Los Angeles, CA, USA
Figure: Differences between and within treatment groups over the 24-week study period are depicted for A, 4 & 9, and B, 5 & 6.
Background: Semaglutide, a GLP-1 receptor agonist, is a highly effective medication for decreasing weight and weight complications by suppressing appetite, improving insulin signaling, and reducing intrahepatic triglycerides (IHTG). However, concomitant loss of muscle mass often accompanies weight loss, which may have consequences on muscle function. The purpose of this analysis was to examine changes in muscle mass, quantity, and function among people with HIV (PWH) treated with semaglutide for metabolic-associated steatotic liver disease (MASLD).
Methods: We leveraged data from the SLIM LIVER (ACTG AS371) study, a single-arm pilot of the effects of semaglutide on IHTG in PWH with MASLD. Participants received subcutaneous semaglutide for 24 weeks (titrated to 1 mg/week by week 4). Poosas volume/fat fraction were assessed from liver magnetic resonance imaging and physical function by 10-time chair rise test and 4m gait speed, at baseline and week 24. Mean change from baseline was estimated with linear regression modeling and associations with Spearman’s correlations.
Results: 51 PWH enrolled; muscle measures were available from 46 participants. The mean age was 50 (standard deviation [SD] 11) years and BMI 35.5 (5.6) kg/m².
43% were women, 33% Black, and 39% Hispanic/Latino. Poas muscle volume decreased by 9.3% (95% confidence interval [CI]: -13.4, -5.2; p<0.001) with an overall mean weight loss of -7.8 kg (CI: -9.5, -6.2) over 24 weeks. Decreases in poas volume were greatest among PWH >60 years old (12.6% [CI: -32.4, -13.3]) vs -7.9% (CI: -12.3, -3.4) in 40-60 and -2.4% (CI: -11.9, 7.2) in <40). No sex differences were observed. Reductions in poas volume (%) correlated with decreases in HbA1c (p=-0.32, p=0.028), BMI (p=-0.31, p=0.038), HbA1c (p=-0.39, p=0.007), and reduction in absolute volume was associated with reduction in fasting triglycerides (p=-0.33, p=0.027). Poas muscle fat decreased by 0.62% (CI: -1.0, 0.17, absolute change), chair rise time improved by 0.73 seconds (CI: -1.4, 2.9) and gait speed improved by 0.05 m/sec (CI: 0.01, 0.10), though these changes did not reach statistical significance (p=0.078). The prevalence of slow gait speed (<1 m/sec) decreased from 63% to 46% (p=0.029).

Conclusion: In PWH using low-dose semaglutide for MASLD, muscle volume decreased, similar to volume changes seen in weight-loss interventions among overweight populations. The observed average improvement in muscle function suggests a beneficial effect of semaglutide on overall muscle quality.

800 A Combination of Steatosis-Fibrosis Index Predicts Major Cardiovascular Events in PLHIV
Maria Luisa Montes, Carmen Busca, Antonio Oliveira, Jose I. Bernardino, Luz Martin-Carbonero, Marta Abadía, Eulalia Valencia, Rafael Mico, Roque Montejano, Rosa De Miguel Buckley, Jose R. Arribas, Juan González-García La Paz University Hospital, Madrid, Spain

Background: Metabolic dysfunction-Associated Steatosis Liver Disease (MASLD) is a high prevalent condition in people living with HIV (PLHIV) and cardiovascular events appear to depend on the presence of advanced stages of MASLD. Indeed, the underlying insulin resistance and liver fibrosis have demonstrated complex interlinked connections affecting cardiovascular damage evolution. Our objective is to analyze the usefulness of two easy-to-use surrogate biomarkers of insulin resistance and liver fibrosis in the prediction of major cardiac events (MACE) in PLHIV.

Methods: A retrospective cohort of PLHIV receiving ART with metabolic disorders (metabolic syndrome, DM2, arterial hypertension, and obesity) and clinically suspected MASLD was studied. We recorded all MACE occurring during follow-up, and we calculated TyG and FIB-4 indexes from the blood results performed at the initial visit in the cohort. TyG>4.68 was considered as severe insulin resistance risk. FIB-4 cut-off values considered for significant fibrosis were adjusted by hepatitis C virus (HCV) coinfection background: >1.3 for non-HCV, >1.45 for HCV coinfected. TyG>4.68 plus FIB-4>1.3/1.45 was considered a risk-combination-index. Cox models were developed to evaluate the effect of risk-combination-index in the prediction of MACE.

Results: We studied 370 subjects with a median follow-up time of 175 (131-191) months. Main baseline characteristics are shown in table 1. HCV-ab were positive in 25.9% of subjects, all of them were treated and had undetectable HCV RNA during follow-up. A total of 19 MACE (8 myocardial infarction, 6 stroke, 5 peripheral arterial disease) were registered. Along follow-up 39% of subjects developed metabolic syndrome, 37% hypertension and 17.6% DM2. TyG>4.68 was found in 56% of subjects and FIB-4>1.3/1.45 in 29.5%, these proportions were adjusted by age, HIV infection duration and HCV coinfection showed that risk-combination-index was associated with MACE: HR 2.6 (CI95% 1.02-6.6; p=0.04). Cox models were adjusted by hepatitis C virus (HCV) coinfection background: > 1.3 for non-HCV, >1.45 for HCV coinfected. TyG>4.68 plus FIB-4>1.3/1.45 was considered as risk-combination-index. Cox models were developed to evaluate the effect of risk-combination-index in the prediction of MACE.

Conclusion: In PWH using low-dose semaglutide for MASLD, muscle volume decreased, similar to volume changes seen in weight-loss interventions among overweight populations. The observed average improvement in muscle function suggests a beneficial effect of semaglutide on overall muscle quality.

801 T-Cell Subsets and Incidence of Diabetes in Persons With HIV in the ACTG Study A5001
Katherine Tassiopoulos1, Junling Wu2, Robert C. Kalayjian1, Susan L. Koletar1, Frank Palella1, John Koethe1, Alan Landay1
1Harvard Chan School of Public Health, Boston, MA, USA, 2MetroHealth Medical Center, Cleveland, OH, USA, 3The Ohio State University, Columbus, OH, USA, 4Northwestern University, Chicago, IL, USA, 5Vanderbilt University, Nashville, TN, USA, 6Icahn University, Chicago, IL, USA

Background: Persons with HIV (PWH) have persistent changes in T cell subsets that can also be modified by aging, and may contribute to the high burden of comorbid conditions, including type 2 diabetes (DM), in older PWH. While studies in persons without HIV have found few associations between T cell subsets and DM or pre-DM, a recent study of US veterans with HIV found higher frequencies of effector memory and senescent CD4+ T cells associated with incident DM. Here, we examined associations of T cell subsets measured at a uniform time post-ART initiation with incident DM among PWH in the AIDS Clinical Trials group study A5001.

Methods: DM was defined as: 2 consecutive non-fasting glucose >200 mg/dl or fasting glucose >126 mg/dl; DM diagnosis; or oral antidiabetic or insulin use for >30 days. We used Cox proportional hazards models to evaluate associations of T cell subsets measured 1 yr post-ART with incident DM. PWH with prevalent DM (≤ 1yr post-ART) were excluded. Multivariable models included age at ART start, sex at birth (gender not available), self-reported race/ethnicity, smoking status, time-updated BMI, triglycerides, and history of hypertension. We explored effect modification by demographic and clinical factors by including interaction terms between each T cell subset and effect modifier in multivariable models.

Results: 1015 PWH had ≥1 subset (% activated CD4 or CD8 [HLA-DR+/CD38+], % senescent CD4 or CD8 [CD28−], % memory CD4 [CD45RA−/CD69+], % naive CD4 [CD45RA+/CD69−]) measured 1yr post-ART. Most (82%) participants were male; 48% White non-Hispanic, 31% Black non-Hispanic, and 21% Hispanic/other ethnicity. Median age was 36 years (Q1,Q3=31,43). There were 62 DM events. PWH with DM were older, had higher triglycerides, higher BMI, and were more likely to have hypertension or low CD4/CD8 ratio than those without DM. PWH in the lowest quartile of % naive CD4 cells had a higher incidence of DM than those in higher quartiles (Figure). The association for lowest vs highest quartiles (combined) was observed in PWH <45 years (ahr=2.08 [95% CI=1.00,4.34] but not ≥45 years (ahr=1.17, 95% CI=0.44,3.11), though the interaction by age was not significant (p=0.32). There were no associations with other T cell subsets and DM.

Conclusion: A lower proportion of naive CD4+ cells, potentially reflective of reduced naive cell replenishment or increased memory cell cell inflation, may be associated with increased diabetes incidence in PWH, particularly among younger persons.

802 Subcutaneous Adipose Tissue Myofibroblasts Are Associated With The Lipidome
Vanderbilt University Medical Center, Nashville, Tennessee

Background: Dyslipidemia is common in persons with HIV (PWH) and mechanistically linked to the development of metabolic disease. Higher plasma triacylglycerides (TG) are associated with the diabetes, while an inverse relationship is observed for sphingomyelin (SM) and phosphatidylcholine (PC) species. Adipose tissue has a critical role in regulating lipid homeostasis, but how adipose tissue cellular composition might influence circulating lipid classes in PWH is unknown. We hypothesized that greater pro-inflammatory
macrophage and pro-fibrotic stromal cells in subcutaneous adipose tissue (SAT) are associated with higher circulating TG and lower SM and PC species.

**Methods:** We performed single-cell RNA sequencing on SAT biopsies from PWH on contemporary antiretroviral therapy with virologic suppression and a range of metabolic fitness. We characterized SAT cell types except adipocytes, which are poorly captured with this method. We simultaneously performed untargeted lipidomic liquid chromatography-high resolution tandem mass spectrometry on plasma samples. We assessed the relationship of cell composition with normalized summed-lipid class intensities with partial Spearman’s adjusted for statin use, sex, body mass index (BMI) and diabetes status.

**Results:** A total of 55 participants were included in this study (non-diabetic=19, prediabetic=18, diabetic=18). The median age was 49 years, BMI 31.5 kg/m², 73% male, 49% White, and 62% treated with an integrase strand transfer inhibitor-based regimen. A higher proportion of myofibroblasts (pro-fibrotic cells) was positively associated with TG (p=0.41, p=0.006) and oxidized TG (OxTG; p=0.39, p=0.002) levels, and inversely associated with SM (p=0.27, p=0.03) and plasmalogen phosphatidylethanolamines (plasmalgen-PE) (p=0.44, p=0.004) but not PC (p=0.22, p=0.11) (Figure 1). Higher intermediate macrophages (IMs) were inversely associated with plasmalgen-PE (p=0.36, p=0.04). The proportion of lipid-associated macrophages (LAMs) was not associated with any lipid class.

**Conclusion:** In PWH, higher proportion of SAT myofibroblasts was moderately associated with higher TG and OxTG, and inversely related to SM and plasmalgen-PE. IMs and LAMs were not significantly associated with these classes except IMs and plasmalgen-PE. These results suggest that a pro-fibrotic composition of non-adipocyte stromal cell types is related to circulating lipid profiles. Future studies using single nuclei RNA-sequencing will be necessary to evaluate the contribution of adipocyte populations.

**Figure 1.** Heatmap of partial Spearman correlation between lipid classes and myofibroblast or macrophage proportion, adjusted for statin use, sex, body mass index, and diabetes status. For intermediate (IM) and lipid-associated macrophages (LAM), n = 50 individuals contributed.

*P = 0.05*

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### 803 Dolutegravir Targets the Hypothalamus to Suppress Energy Expenditure via Estrogen Receptor

**Ikrak Jung**, Sunghen Jin, Woosub Yang, Yoomin Cho, Becky Tu-Sekine, Frederick Anokye-Danso, In-Hyun Park, Todd T. Brown, Sangwon F. Kim

*The Johns Hopkins University School of Medicine, Baltimore, MD, USA, *Yale University, New Haven, CT, USA*

**Background:** Antiretroviral therapy (ART) containing integrase strand transfer inhibitors (INSTI) has been associated with weight gain in both ART-initiation and switch studies, especially in women, but the underlying mechanisms are unclear. Estrogen promotes energy expenditure via suppressing AMPK-dependent kinase (AMPK) in the hypothalamus and yet few studies are available examining the central effects of INSTI on weight gain. Hence, we hypothesized that dolutegravir (DTG) may inhibit thermogenesis via estrogen receptors in the hypothalamus.

**Methods:** We examined the effects of DTG (10mg/kg for 5 days) on food intake, energy expenditure, oxygen consumption in female mice using the Comprehensive Laboratory Animal Monitoring System. Adipose and brain tissues were analyzed using qRT-PCR and immunoblotting for appetite and thermogenesis. Primary hypothalamic neurons and inducible human pluripotent stem cells-driven hypothalamic organoids were treated with DTG and estradiol and examined for changes in cellular signaling associated with the regulation of energy homeostasis. Computational analysis was performed to evaluate the potential interaction between DTG and estrogen receptors.

**Results:** We found that DTG administration to female mice reduced oxygen consumption and energy expenditure by 16% without affecting food intake. Gene expression analyses in adipose tissues confirmed that thermogenic marker expression (UCP1, Dio2 and Cidea) was reduced. Moreover, DTG administration activated the AMPK signaling cascade in the hypothalamus. Murine primary hypothalamic neurons treated with estrogen led to inactivation of AMPK while DTG attenuated estrogen-mediated suppression of AMPK activity. We further confirmed the DTG activates AMPK and inhibits estrogen effect on human hypothalamic organoids, which retain the heterogeneity and neural circuitry of the brain. Finally, molecular docking analysis showed that DTG can physically bind to estrogen receptors.

**Conclusion:** DTG administration increased body weight by suppressing energy expenditure without affecting food intake. Tissue analyses revealed that DTG activates the hypothalamic AMPK pathway, which suppresses thermogenesis. In vitro study using murine primary hypothalamic neurons and human hypothalamic organoids showed that DTG inhibits estrogen-mediated hypothalamic regulation of thermogenesis. These findings suggest a novel mechanism by which INSTIs may lead to weight gain, especially in women. The figure, table, or graphic for this abstract has been removed.

### 804 Weight Gain After Initiating ART Close to HIV Seroconversion: Is There a Return to Health Effect?

**Nikos Pantazis**, Sophie Grabar, Marc Van der Valk, Caroline Sabine, Imma Jarrin, Laurence Meyer, Christina Carolander, John Gill, Shema Tarig, Alain Volny Anne, Fiona Burns, Elisa Ruiz-Burga, Giota Touloumi, Kholoud Porter, for the CASCADE Collaboration

1 National and Kapodistrian University of Athens, Athens, Greece, 2 Institut National de la Santé et de la Recherche Médicale, Paris, France, 3 Stichting HIV Monitoring Foundation, Amsterdam, Netherlands, 4 University College London, London, United Kingdom, 5 Institute of Health Carlos III, Madrid, Spain, 6 Université Paris-Sud, Paris, France, 7 Karolinska University Hospital, Stockholm, Sweden, 8 Southern Alberta Clinic, Calgary, Canada, 9 Paris, France

**Background:** Findings from seroprevalent cohorts suggest that weight gain after ART initiation is greater with regimens containing integrase strand transfer Inhibitors (INSTI) than with other antiretrovirals. We investigate weight trends among individuals initiating ART within one year of seroconversion (SC).

**Methods:** Included individuals from the CASCADE Collaboration seroconverted 1997-2022, were ≥16 years, had HIV- to HIV+ test interval ≤1 year or other laboratory evidence of SC, and initiated ART ≤12 months from SC. Weight changes from baseline (censored after switching ART class) were analyzed with piecewise linear mixed models.

**Results:** 6482 (22.7%) of 28566 individuals were included (2814, 1999 and 1669 with INSTI, boosted PI or NNRTI regimens, respectively). Most acquired HIV through sex between men (79.3%), and 8.3% men and 8.9% women through heterosexual contact. Median (IQR) age at SC was 34 (27, 43) years, CD4 at ART initiation 451 (230, 620) cells/μl and follow-up time 1.4 (0.3, 3.6) years. Weight changes differed significantly (p<0.001) by ART class and baseline BMI. In the first 6 months, weight gains were generally most pronounced among those on NNRTIs, regardless of baseline weight. This trend remained for 3 years for BMI categories <30, although those receiving boosted PIs also experienced weight gain. “Typical individuals” (Figure) had significant weight gains with all three ART classes after 3 years except for baseline BMI ≥30. Individuals with BMI 18.5-29.9 gained weight significantly faster with INSTIs compared to other ART classes. Overall, 16% with BMI 18.5-24.9 and 11% with BMI 25-29.9 on INSTIs gained >10% of their baseline weight after 3 years (9% and 8% for boosted PIs; 7% and 6% for NNRTIs). Significant differences in initial (0-6 months) rate of weight gain were observed between INSTIs (p<0.001). Bictegravir or Elvitegravir + TAF backbone were associated with the fastest rates. Dolutegravir without TAF/TDF, and Elvitegravir + TDF were associated with the slowest. Estimated (95% CI) weight gains after 3 years among “typical individuals” with BMI 18.5-24.9 were 5.6 (4.5, 6.6), 4.4 (3.5, 5.4), 3.6 (2.8, 4.4), and 3.4 (2.5, 4.2) kgs, respectively.

**Conclusion:** As reported in seroprevalent cohorts, Bictegravir and Elvitegravir combined with TAF were associated with the fastest increases in weight. Given that ART was initiated soon after SC, it is unlikely that this is a return to health phenomenon, although the effect of unmeasured confounders can’t be disregarded.
Switching to Integrase Strand Transfer Inhibitors and the Risk of Diabetes in Persons With HIV

Y. Joseph Hwang, Catherine Lesko, Todd T. Brown, Jeanne C. Keruly, LaQuita N. Snow, Jarratt D. Pytel, Olusunse Faye-Adewumila, Richard D. Moore, Anthony FejzI, and John S. Nuttall

The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Background: Integrase strand transfer inhibitors (INSTIs) are commonly used antiretroviral therapy (ART) among people with HIV (PWH). INSTI use is associated with weight gain, hyperglycemia, and diabetes among ART-naive PWH. We estimated the risk of incident diabetes associated with switching from a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI) to an INSTI.

Methods: We studied PWH aged ≥18 years in the Johns Hopkins HIV Clinical Cohort. PWH with no history of diabetes who had used NNRTI- or PI-based ART for ≥180 days were followed from attended HIV primary care visits between 2007 and 2022 until incident diabetes, last clinical encounter, or December 31, 2022. PWH who switched to bicitravir, dolutegravir, elvitegravir, or raltegravir were assigned to the INSTI group and PWH who were continued on an NNRTI or PI were assigned to the non-IN INSTI group. Incident diabetes was defined as a laboratory hemoglobin A1c value ≥6.5%, initiation of diabetes-specific ARV, especially with integrase inhibitors (INSTI) and tenofovir alafenamide (TAF).

Results: Our sample included 2,354 PWH, of whom 891 PWH switched to an INSTI and contributed an encounter to the INSTI group, 2,293 PWH contributed ≥1 encounters to the non-IN INSTI group. The median age was 49 years and 66% were male. Switching to any INSTI was not statistically significantly associated with weight gain, hyperglycemia, and diabetes among ART-naive PWH. We estimated the risk of incident diabetes associated with switching from a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI) to an INSTI.

Conclusion: Switching from an NNRTI or PI to INSTI-based ART did not appreciably increase the risk of incident diabetes. These findings can inform antiretroviral prescribing in the common clinical scenarios, where switching to INSTI-based therapy is considered in the care of PWH.

Table 1: Risk of incident diabetes associated with switching to integrase strand transfer inhibitors (INSTIs) versus continuing on non-nucleoside reverse transcriptase inhibitor (NNRTIs) or protease inhibitor (PIs)

<table>
<thead>
<tr>
<th>ART Group</th>
<th>Number of PWH</th>
<th>Number of events</th>
<th>Incidence rate (per 1,000 person-years)</th>
<th>Adjusted hazard ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNRTI (2,293)</td>
<td>268 (10.8)</td>
<td>20.8</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Any INSTI (991)</td>
<td>121 (13.8)</td>
<td>26.9</td>
<td>1.09 (0.57–2.49)</td>
<td></td>
</tr>
<tr>
<td>Bicitravir (96)</td>
<td>7 (7.3)</td>
<td>55.4</td>
<td>1.24 (0.53–2.35)</td>
<td></td>
</tr>
<tr>
<td>Dolutegravir (50% of 216)</td>
<td>49 (11.3)</td>
<td>28.9</td>
<td>0.94 (0.42–2.14)</td>
<td></td>
</tr>
<tr>
<td>Elvitegravir (206)</td>
<td>28 (12.0)</td>
<td>25.4</td>
<td>1.08 (0.65–1.88)</td>
<td></td>
</tr>
<tr>
<td>Raltegravir (230)</td>
<td>47 (17.5)</td>
<td>24.4</td>
<td>1.15 (0.88–1.50)</td>
<td></td>
</tr>
</tbody>
</table>

Data are given as mean (standard deviation).

TDF = Tenofovir disoproxil fumarate
TAF = Tenofovir alafenamide

Weight Change After Starting Doravirine Among ART-Experienced Individuals in the US

Karam Mounzer1, Laurence Brunet2, Michael Sension3, Ricky K. Hsu4, Jennifer S. Fusco5, Yohance O. Whiteside4, Gregory P. Fusco4

1Philadelphia FIGHT, Philadelphia, PA, USA, 2Epiphany, Raleigh, NC, USA, 3CAN Community Health, Sarasota, FL, USA, 4 AIDS Healthcare Foundation, New York, NY, USA, 5Emck Co & Inc, Rahway, NJ, USA

Background: Weight gain has been associated with the use of antiretrovirals (ARV), especially with integrase inhibitors (INSTI) and tenofovir alafenamide (TAF), but less so with non-nucleoside reverse transcriptase inhibitors (NNRTI).

In 2018, doravirine (DOR) became the latest NNRTI to be approved. We assessed
Changes in weight over time after starting DOR among virologically suppressed individuals.

**Methods:** From the US-based OPERA cohort, ART-experienced adults with HIV who started a DOR-based regimen between 30AUG2018-30NOV2022 with a viral load <50 copies/mL were included (followed through 31MAY2023). Univariate linear mixed models were used to estimate rates of weight change on DOR; restricted cubic splines on time provided flexibility. Results were stratified by sex. Two sensitivity analyses were conducted to account for use of other ARVs before and after DOR start (a) restriction to those who maintained the same INSTI-TAF combination (b) stratification by efavirenz (EFV)-tenofovir disoproxil fumarate [TDF] use.

**Results:** Of 388 included individuals, 79% were men, 33% were Black, and 78% were overweight or obese (BMI ≥25 kg/m²) at DOR start. Most regimens prior to DOR start included an INSTI with TAF (47%) or an INSTI without TAF (31%); 16% included TAF without an INSTI and 7% included neither INSTI nor TAF. DOR was combined with both INSTI and TAF (31%), with INSTI without TAF (30%), with TAF without INSTI (11%) or with neither (28%). Overall, people starting DOR lost a statistically significant average of 0.80 kg/year (95% CI: -1.32, -0.28). Both women and men experienced a statistically significant weight loss; women (70% Black) lost weight at a rate of -1.67 kg/year (95% CI: -3.32, -0.02), and men at a rate of -0.60 kg/year (95% CI: -1.12, -0.08). Among those who had the same INSTI-TAF combination throughout, there was a statistically non-significant trend toward weight loss. When EFV and TDF were absent both before and after DOR start, DOR was statistically significantly associated with weight loss.

**Conclusion:** In one of the first real-world analyses of weight changes among virologically suppressed individuals who started a DOR-based regimen in the US, DOR was associated with a modest but statistically significant weight loss overall. Weight loss in women is of particular significance given that weight gain has often been associated with female sex. These findings are clinically meaningful given that most individuals included were overweight or obese at DOR start.

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**Predictors of Weight Gain in the ADVANCE, NAMSAL, and WRHI trials:**

**EFV, TDF, and Baseline CD4 Count**

Andrew Hill, Bryony Simmons, Francois Venter, Alexandre Calmy, Eric Delporte, Tamara Tovar-Sanchez, Charles Kousanack, Mireille Mpoudi-Enyeame, Godspower Akpomiamie, Bronwyn Bosch, Simo Sokhela

1. University of Liverpool, Liverpool, United Kingdom
2. Health Economics and Epidemiology Research Office, Johannesburg, South Africa
3. Wits Reproductive Health and HIV Institute, Johannesburg, South Africa
4. University Hospitals of Geneva, Geneva, Switzerland
5. University Hospital Montpellier, Montpellier, France
6. Centre Hospitalier Universitaire de Montpellier, Montpellier, France
7. Central Hospital of Yaoundé, Yaoundé, Cameroon

**Background:** Weight gain is common during first-line ARV treatment, especially among women and those of black race. Use of TDF or EFV can suppress viral load <50 copies/mL but this might be from regaining weight lost during advanced disease.

**Methods:** Data were pooled from three clinical trials: ADVANCE (n=1053), NAMSAL (n=624), and WHRI001 (n=536). These randomised trials evaluated first-line ARV regimens (TAF/XTC/DTG, TDF/XTC/DTG, and TDF/XTC/EVF) in Cameroon and South Africa. BMI over 96 weeks was analysed, stratified by baseline CD4 count as a marker for disease stage (<100, 100-200, 200-350, ≥350 cells/μL). Multivariate models at week 96 assessed factors associated with BMI and clinical obesity (BMI ≥30), adjusting for baseline CD4 category, age, sex, TDF, and clinical trial.

**Results:** At baseline, mean age was 34.5 (SD 8.9), 60% were female, 14% had CD4 <100 cells/μL, and 31% had CD4 ≥350. Lower baseline BMI was strongly correlated with lower baseline CD4 count (p<0.001). At week 96, mean unadjusted BMI change was highest in the <100 CD4 group (+3.2 kg/m²; SD 3.1) and lowest in the CD4≥350 group (+1.1; SD 2.4). Individuals with advanced disease on TAF-based regimens experienced greater BMI increases compared to those on TDF-based regimens (Figure 1). For participants on TAF-based treatment (ADVANCE only), increases in BMI to Week 96 were significantly higher in people with CD4 <100 (+5.0, SD 3.1) compared to the ≥350 group (1.6, SD 2.2). In the adjusted model, for people taking TAF/FTC/DTG, BMI at Week 96 was significantly higher for people with baseline CD4 <100 (28.4 [95%CI 26.7-30.1]) compared to CD4≥350 (25.3 [95%CI 24.4-26.3]; p = 0.001). However, on TDF or EFV-based regimens, there was no difference in BMI across the CD4 categories. Analyses using clinical obesity (BMI >30 kg/m²) showed consistent results: people taking TAF/FTC/DTG with CD4 <100 were significantly more likely to become obese after 96 weeks of first-line treatment.

**Conclusion:** For people taking TAF/FTC/DTG, baseline CD4 <100 cells/μL at treatment initiation was associated with significantly higher BMI and clinical obesity at Week 96. Weight continued to rise over time for people with low CD4 counts taking TAF/FTC/DTG, above the levels seen with higher baseline CD4 counts. Use of TDF and EFV were associated with smaller rises in weight. Effective weight management is required with current regimens to avoid complications associated with significant weight increases.

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**The Relationship Between Plasma Oxylipins and INSTI-Associated Weight Gain in Women Living With HIV**

Chin-An Yang, Cyra C. Mehta, Qian Yang, Tsungirirai Mambaza, Jhgo Ofotokun, Kehmia Titani, Anandi N. Sheth, Kristal M. Maner-Smith, Thomas R. Ziegler, Cecile D. Lahiri, Jessica A. Alvarez

Emory University, Atlanta, GA, USA

**Background:** Initiating or switching to integrate strand-transfer inhibitors (INSTIs) for HIV is associated with body weight gain, particularly in women. However, the specific mechanisms driving this effect remain unclear. Previous findings have implicated omega-6-derived poly-unsaturated fatty acids (PUFAs) as potential mediators of increased adiposity in people without HIV. This study investigated the relationship between INSTI-associated weight gain and plasma oxylipins, downstream PUFAs metabolites, in women living with HIV (WLH) by utilizing a targeted lipidomics approach in a longitudinal design.

**Methods:** Virologically suppressed (<200 c/mL) WLH from the Atlanta Women’s Interagency HIV Study (WIHS) on antiretroviral therapy were grouped based on INSTI usage and weight change during the follow-up period (weight gain defined as ≥5% change, or weight maintenance as <5% change from baseline). We leveraged stored blood samples collected at three time points: 0-12 months before switching to or adding INSTI (baseline), 1-6 months post switch/add, and 1-2 years post switch/add, with comparable time points in the non-INSTIT group. Targeted lipidomics assessed 40 oxylipins via liquid chromatography-mass spectrometry, and differences between groups assessed using linear mixed models.

**Results:** Sixty women, aged 28-62 years, were included (n=33 INSTI, n=27 non-INSTIT) with n=15 weight gainers in the INSTI group and n=9 weight gainers in the non-INSTIT group. Fifty-six women identified as Black, 3 as White, and 1 as Hispanic. Within the INSTI group, nine oxylipins differed between weight gainers and maintainers over time (p-value < 0.05, Figure). Three oxylipins, linoleoyl ethanolaminole (LEA), arachidonyl ethanolaminole (AE), and 9-HpODE also differed between weight gainers and maintainers among women in the non-INSTIT group. Six oxylipins were unique to those who switched to INSTI, including α-linolenoyl ethanolaminole (ALAE), palmitoyl ethanolaminole (PEA), oleoyl ethanolaminole (OEA), 9-HODE, 9-HOTrE, and prostaglandin E2 glycerol ester (PGE2-G).

**Conclusion:** Employing a targeted lipidomics approach, six oxylipins were linked to INSTI-associated weight gain in WLH. Among these oxylipins, ALAE and OEA have been previously implicated in weight gain processes in healthy adults, while 9-HODE and 9-HOTrE have been linked to weight gain in mouse models. Further research is needed to identify oxylipin profiles unique to INSTI usage and to determine potential underlying biological mechanisms for INSTI-associated weight gain.
811 Dolutegravir, Body Mass Index, and Metabolic Syndrome in the IeDEA Sentinel Research Network

Samir K. Gupta1, Susan Ofner1, Beverly Musil1, Constantin Yianoutsos1, Gillies Wandel1, Belinda Chihota2, Albert Minga3, Ephram Mensah3, Vidyia Mave4, Awachana Jiamusuk1, Brenda E. Crabtree-Rimbez1, Rodrigo C. Moreira5, Suzanne Goodrich6, Aggrey Semere7, for the Sentinel Research Network of IeDEA

1Indiana University, Indianapolis, IN, USA, 2University of Born, Bern, Switzerland, 3Center for Infectious Research in Zambia, Lusaka, Zambia, 4Centre Médical de Suivi des Donneurs de Sang, Abidjan, Côté d’Ivoire, 5Epsom Vie - Togo, Lomé, Togo, 6Byramjee Jeejeebhoy Government Medical College, Pune, India, 7University of New South Wales, Darlinghurst, Australia, 8Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, 9Instituto Nacional de Infectología Eunice Chagoya, Rio de Janeiro, Brazil, 10Makerere University College of Health Sciences, Kampala, Uganda

Background: Dolutegravir (DTG) use has been associated with increased weight compared to efavirenz in many international settings. As such, we hypothesized that DTG is also associated with greater cardiometabolic risk globally.

Methods: We performed a cross-sectional study examining associations between DTG use, body mass index (BMI), and metabolic syndrome (MetS), as defined by the International Diabetes Federation) using baseline data of the IeDEA Sentinel Research Network (SRN) cohort study. The SRN prospectively collected data from people with HIV from low- and middle-income sites worldwide, aged ≥40 years, and on ART for at least six months at time of enrollment. Using multivariable linear and logistic regression models, respectively, BMI and MetS were assessed using independent variables of DTG use vs. non-use at enrollment, age, sex, country, smoking status (ever vs. never), and HIV RNA level (<200 vs. ≥200 c/ml). CD4 cell count was not included as it was unavailable in some countries. We assessed the potential for interaction by sex by country.

Results: 1,446 participants from Brazil (N=212), Côte d’Ivoire (N=298), India (N=188), Kenya (N=77), Mexico (N=193), Togo (N=244), Uganda (N=100), and Zambia (N=134) were included. Overall, 54% were female, 94% had HIV-1 RNA <200 c/ml, 53% were using DTG (78% for >6 mos), and median age was 50.5 years. Median BMI was 25.3 kg/m², and 35% had MetS. DTG use and younger age were significantly associated with higher BMI (Table), with a significant interaction by sex by country. In India, females and males had similar BMI; in other countries BMI was similar among females and varied in males (higher in Brazil and Mexico in low and in Kenya, in Uganda, and Zambia). MetS was significantly associated with older age and again with significant interaction by sex by country. Compared to males, females had higher odds (adjusted OR (95% CI)) of MetS in Brazil (20.75 (2.71, 158.97)), Uganda (15.17 (4.69, 49.11)), Kenya (12.53 (2.55, 61.69)), Togo (4.31 (1.98, 9.36)), Côte d’Ivoire (3.21 (1.80, 5.74)), and Mexico (3.02 (1.18, 7.72)), but not in Brazil or India. No other interactions involving DTG use, sex, and country were associated with BMI or MetS.

Conclusion: DTG was associated with modestly higher BMI but not with MetS in the IeDEA SRN cohort. These data suggest cardiometabolic risk varies across low- and middle-income settings and is dependent on age, sex, and country.

Table. Multivariable results of body mass index and metabolic syndrome in the IeDEA SRN.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate (95% CI)</th>
<th>P value</th>
<th>OR estimate (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (categorical)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI &lt;20 vs. ≥20</td>
<td>1.13 (1.04, 1.23)</td>
<td>0.004</td>
<td>1.13 (1.04, 1.23)</td>
<td>0.004</td>
</tr>
<tr>
<td>MetS (categorical)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MetS vs. no MetS</td>
<td>0.59 (0.43, 0.82)</td>
<td>0.003</td>
<td>0.59 (0.43, 0.82)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

812 TDF and Efavirenz but Not INSTI or TAF Use Are Associated With Weight Gain During cART

Henning J. Drechsler1, Ammeris Luque1, Ikwo Oboho1, John Hanna2, Christopher Clark2, Ngozi Enwerem3, Roger Bedimo2, Samir K. Gupta1, Beverly Musil1, Constantin Yianoutsos1, Gillies Wandel1, Belinda Chihota2, Albert Minga3, Ephram Mensah3, Vidyia Mave4, Awachana Jiamusuk1, Brenda E. Crabtree-Rimbez1, Rodrigo C. Moreira5, Suzanne Goodrich6, Aggrey Semere7, for the Sentinel Research Network of IeDEA

1Indiana University, Indianapolis, IN, USA, 2University of Born, Bern, Switzerland, 3Center for Infectious Research in Zambia, Lusaka, Zambia, 4Centre Médical de Suivi des Donneurs de Sang, Abidjan, Côté d’Ivoire, 5Epsom Vie - Togo, Lomé, Togo, 6Byramjee Jeejeebhoy Government Medical College, Pune, India, 7University of New South Wales, Darlinghurst, Australia, 8Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, 9Instituto Nacional de Infectología Eunice Chagoya, Rio de Janeiro, Brazil, 10Makerere University College of Health Sciences, Kampala, Uganda

Background: Combination antiretroviral therapy (cART) containing integrase strand transfer inhibitors (INSTIs) and/or tenofovir alafenamide (TAF) has been associated with greater weight gain (WG) than cART without these drugs. Yet few studies have adjusted for multiple individual antiretrovirals (ARVs) and for both anchor and backbone ARV component.

Methods: We studied WG in cART-naïve patients by analyzing body mass index (BMI) change every 3 months for 3 years after cART initiation in a large HIV Clinic in the Southern US. From 2008-2022 we studied all patients who initiated cART with either dolutegravir (DTG), bictegravir, elvitegravir, raltegravir, atazanavir, darunavir (DRV), ritonavir (RTV), or efavirenz (EFV) as exclusive anchor drug if used in combination with either TAF, tenofovir disoproxil fumarate (TDF), or..
abacavir (ABC) as backbone. We used multi-variable generalized estimating equations (GEE) to assess the association between WG and individual ARV use in the anchor and backbone category. Within each category, patients were censored if they stopped, switched, or added another ARV. We adjusted for main effects of HIV-related, demographic, substance use, and clinic utilization parameters, in addition to time, and retained only significant covariates and factors for the final model.

Results: 4,194 patients contributed 6,514 patient-years and 20,528 BMI measurements. The majority were black (55%), male (77%), and non-Hispanic (72%). Median baseline BMI was 24.4, inter-quartile range (IQR) 21.6-28.2. After 3-years, median BMI was 27.1 (IQR 23.8-31.3), and median BMI gain was 1.8 (IQR 0.2-4.1). The most used ARVs were DTG (23%), EFV (22%), and DRV (14%) in the anchor, and TDF (58%), TAF (21%), and ABC (21%) in the backbone group. In the final model, within the anchor group, we found no significant WG differences in pairwise comparisons between any of the INSTIs, RIL, or protease inhibitors; the same was true for ABC and TAF in the backbone group. In contrast, both EFV and TDF were associated with significantly lower WG in all pairwise comparisons within their respective groups and were retained for the final model shown in the table. Calendar year, annual follow-up frequency, and Hispanic ethnicity did not significantly contribute to BMI change.

Conclusion: Over a 15-year period, our demographically diverse patient population experienced substantial WG in the first 3 years after cART initiation. Among 11 examined ARVs, only EFV and TDF were independently associated with (decreased) WG.

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813 Lipid Composition and Weight Changes at 48-week 3TC-DTG and FTC/TAF/BIC: Data of the ICONA Cohort

Robert Rovito1, Valeria Bonomo Camilla Tincati2, Matteo Augello3, Alessandro Tavelli3, Alessandra Rodano3, Francesca Bai4, Valentina Mazzotta5, Andrea Antinori6, Eugenia Quirin-Roldan7, Andrea Giacomelli8, Giovanni Guaraldi9, Antonella D’Armimono10, Giulia Marchetti11, for the ICONA Foundation Study Group.

University of Milan, Milan, Italy; Iconca Foundation, Milan, Italy; Lazzar Spallanzano National Institute for Infectious Diseases, Rome, Italy; University of Brescia, Brescia, Italy; Luigi Sacco University Hospital, Milan, Italy; University of Modena and Reggio Emilia, Modena, Italy.

Background: cART start has been associated with weight gain (WG), which entails an increased dysmetabolic risk in PLWH, the biologic correlates of which are ill defined. We assessed WG and lipid profile in cART-naive patients starting INSTI-based dual (DT-3TC-DTG) or triple (TT-FTC/TAF/BIC) cART.

Methods: We performed untargeted lipidomic on PLWH of the ICONA cohort both prior (T0) and 48w after DT or TT cART (T1). Raw data were aligned, normalized, and ions from both modes were merged for multivariate analysis. Supervised regression modelling was performed with Orthogonal Partial Least Squares Discriminant Analysis; significant biomarkers are selected based on variables’ significance in the model (VIP> 1.5), t-test (p<0.05), and fold change (FC>2), followed by pathway enrichment analysis (PEA).

Results: 119 PLWH were included: 62 DT, 57 TT at T0, DT patients were older, more frequently male, with higher CD4, lower HIV-RNA, fewer AIDS diagnoses, higher body weight and HDL, despite comparable total and LDL cholesterol, triglycerides and lipid lowering agents (Fig.1A). At T1, TT showed higher WG versus DT (5.1 ± 5.8 vs 2.2 ± 3.2 kg SD, p<0.001). Both DT and TT displayed a net separation in the OPLS-DA model between T0 and T1, witnessing substantial lipidomic changes (Fig.1B-C). In DT, 109 lipids were significantly different at T1, and 66 lipids in TT, with a higher proportion of up-regulated lipids in TT (83.3% vs 30.3%, p<0.0001) (Fig.1D-E). While both treatments resulted in glycerolipids and glycerophospholipids changes (PEA), DT mainly modified glycerolipids (diacylglycerol-DAG, monoacylglycerol-MG, monoglycerolglycerol-MAG), and TT glycerophospholipids (phosphatidylcholines-PC, lysophosphatidic acids-LPA). When seeking for associations between WG and lipidome, WG positively correlated with PC lipids, and negatively with DAG in TT PLWH only, with no significant correlations detected in DT.

Conclusion: First-line 48-week cART substantially and differentially shapes the plasmatic lipid composition, with 3TC/DTG mainly affecting DAG, and FTC/TAF/BIC the PC pathways. Most interestingly, the correlation between higher WG and glycerophospholipids metabolism with potential phosphatidylcholines involvement in FTC/TAF/BIC, that is not seen in DT, suggests distinct interactions between lipidomic signature and body weight according to cART regimens, that merit consideration when treating PLWH with additional metabolic risk factors. The figure, table, or graphic for this abstract has been removed.

814 Phase 4 DEFINE Switch Study to Manage InStI-related Weight Gain: Metabolics and Biomarker Analysis

Johnnie Lee1, David Anderson2, Nina Ahmad2, Richard B. Simonson3, Ping Xu4, Briana Joumel3, Tien-huei Hsu5.

1Janssen Scientific Affairs, LLC, Titusville, NJ, USA; 2Janssen Research & Development, LLC, Titusville, NJ, USA.

Background: Integrase strand transfer inhibitor (INSTI)—based antiretroviral therapies are associated with greater weight gain than non-nucleoside reverse transcriptase inhibitor—or boosted protease inhibitor (PI)—based regimens, and these effects disproportionately impact Black and Hispanic individuals and women living with HIV-1. DEFINE is the first prospective, randomized trial to explore the impact of switching from an INSTI-based regimen to a PI-based regimen to mitigate or reverse INSTI-related weight gain. As previously reported, the primary Week 24 analysis found no significant difference in percent change in body weight from baseline when switching to darunavir/ cobicistat/etravirine/tenofovir alafenamide (D/C/TAF) compared to continuing INSTI+tenofovir alafenamide (TAF)/emtricitabine (FTC).

Methods: DEFINE (NCT04442377) is a randomized (1:1), prospective, 48-week, active-controlled, open-label, multicenter phase 4 study evaluating switching to D/C/TAF versus continuing INSTI+TAF/FTC in virologically suppressed adults with HIV-1 who had ≥20% weight gain while on the INSTI-based regimen. The primary objective was to assess percent change in body weight from baseline to Week 24. Metabolic and biomarker data through Week 24 are reported in this analysis.

Results: Among the 103 adults who were randomized to D/C/TAF (n=53) or continued INSTI+TAF/FTC (n=50), 30% were female, 61% were Black/African American, and the median BMI was 22.7 kg/m². Consistent with the primary endpoint, at Week 24 most participants remained classified as obese (D/C/TAF: 53%; INSTI+TAF/FTC, 70%) and had experienced minimal BMI and waist circumference changes. Glucose and HbA1c values remained largely unchanged through Week 24; however, there were small decreases in insulin and HOMA-IR values in the INSTI+TAF/FTC arm (Table). No participants in either arm decreased medication dosages or entirely stopped lipid-lowering, anti-glycemic, or anti-hypertensive medications through Week 24. Changes in leptin, adiponectin, and α-melanocyte stimulating hormone were minimal in both arms, as were changes in NAFLD fibrosis score. The percent of participants at high risk of NASH by HAIR score decreased in both arms.

Conclusion: Consistent with the minimal body weight changes observed through Week 24, metabolic and biomarker data remained relatively stable. Metabolic parameters in this high-BMI population did not improve following antiretroviral switch, highlighting that weight gain should be a pretreatment consideration.

Table. Summary of key metabolic parameters and biomarkers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D/C/TAF Baseline</th>
<th>D/C/TAF Week 24</th>
<th>INSTI+TAF/FTC Baseline</th>
<th>INSTI+TAF/FTC Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, median, µU/mL</td>
<td>10.9 (9.0-13.0)</td>
<td>11.7 (9.7-13.7)</td>
<td>12.7 (11.7-13.7)</td>
<td>9.9 (8.0-12.0)</td>
</tr>
<tr>
<td>HOMA-IR, median</td>
<td>2.59 (1.90-3.30)</td>
<td>2.76 (2.20-3.40)</td>
<td>3.49 (2.80-4.30)</td>
<td>2.33 (1.80-3.20)</td>
</tr>
<tr>
<td>Leptin, median, µg/mL</td>
<td>18.8 (13.0-26.6)</td>
<td>22.0 (17.0-29.6)</td>
<td>22.76 (21.0-24.5)</td>
<td>19.72 (18.0-21.5)</td>
</tr>
<tr>
<td>Adiponectin, median, mg/L</td>
<td>3.90 (2.90-5.00)</td>
<td>3.50 (2.90-4.00)</td>
<td>3.60 (2.90-4.50)</td>
<td>3.70 (3.00-4.20)</td>
</tr>
<tr>
<td>α-melanocyte stimulating hormone, median, µg/L</td>
<td>18.0 (16.0-20.0)</td>
<td>16.0 (14.0-18.0)</td>
<td>13.0 (10.0-16.0)</td>
<td>17.0 (15.0-20.0)</td>
</tr>
</tbody>
</table>

815 Weight Gain in People With HIV (PW) vs People Without HIV (PWoH) Over a 3-Year Period

Richard A. Ellen1, Joshua Gruber2, Ianna Radchenko3, Paul E. Sax4, Megan Dunbar5, Joseph J. Ferrie1, Calvin Goh1, Gregory Huhn6, Keri N. Althoff7, Grace A. McComsey1, 2

1Trio Health, Inc, Louisville, CO, USA; 2Geleden Sciences, Inc, Foster City, CA, USA; 3Brigham and Women’s Hospital, Boston, MA, USA; 4University of North Carolina at Chapel Hill, NC, USA; 5Ruth M. Rothstein CORE Center, Chicago, IL, USA; 6The Johns Hopkins University, Baltimore, MD, USA; 7University Hospitals Cleveland Medical Center, Cleveland, OH, USA.

Background: The study evaluated weight (wt) change and shift in BMI class on PWH on antiretroviral therapy (ART) over 3 years (3y) vs PWoH matched on baseline (BL) characteristics.

Methods: Retrospective study using Trio Health HIV Network EMR data from federally qualified health centers in US. Eligibility: ≥18 yrs, in care between 1/1/2015-8/1/2023, with BL and 3y wt measures (all); PWH: treatment-experienced suppressed at BL and 3y or suppressed on 1st ART with BL≥6mo.
since suppression and ≥12mo since ART start. BL characteristics were compared to the lean cohort. The lean cohort exhibited a drastic increase in both CRP and LBP throughout the time course. The ALR was inversely correlated to CRP levels at necropsy.

Conclusion: SIV infection and subsequent ART significantly decrease the ALR in lean animals to values similar to those seen in pre-diabetic and dysmetabolic obese animals. Thus, SIV and ART induce an obese phenotype in initially lean animals. Therefore, weight gain and/or increases in BMI in people living with HIV are not adequate measures as cardiometabolic risks may be independent of these factors.

817 Transcriptionomics and Proteomics Reveal Differential Pathways in DTG/3TC vs 3DR Regimens in PLHIV
Victoria Rios-Vazquez, Wilhelm A. Vos, Marc Blauuw, Louise E. van Eekeren, Albert L. Groenendijk, Quirijn de Mast, Leo Joosten, Mihai Netea, Willem L. Blot, Janneke E. Stalenhoef, Jan van Lunzen, Andre J. van der Ven
1 Radboud University Medical Center, Nijmegen, Netherlands, 2 Erasmus University Medical Center, Rotterdam, Netherlands, 3 OVGU, Amsterdam, Netherlands

Background: Drug toxicity in people living with HIV (PLHIV) using CART is a concern. Nucleoside analogues are associated with mitochondrial damage. Two- drug regimens (2DR), such as DTG (integrase strand inhibitor) and 3TC may reduce drug toxicity, but the molecular effects remain unclear. Systemic effects of DTG/3TC and triple therapies (3DR) were studied using multi-omics.

Methods: Data are used from the 2000HIV (NCT03994835) study that includes discovery (n=1275) and validation (n=212) cohorts of PLHIV with ~11.5 years of CART stratified by treatment regimen. We measured ~2368 plasma proteins (Olink Explore), and ~59347 genes expression by PBMC Bulk RNA-seq. We compared differential gene (DEG) and protein (DEP) expression and enriched pathways between PLHIV using DTG/3TC (n=188) versus 3DR containing INSTI (3DR-INSTI, n=526) or without (3DR-non-INSTI, n=773), and compared 3DR-INSTI versus 3DR-non-INSTI, adjusting for sex, age, ethnicity, and pre-CART conditions.

Results: Discovery cohort data showed limited DEG (5 up, 15 down) and DEP (2 up, 27 down) in DTG/3TC compared to 3DR-INSTI (Figure). DTG/3TC and 3DR-non-INSTI comparison showed 21 DEG (14 down) and 131 DEP (119 down). 3DR-INSTI versus 3DR-non-INSTI revealed no DEG and limited DEP (63 up, 83 down). Gene set enrichment analysis on the total summary statistics of genes and proteins ranked by log2(−p-value) revealed significant results (p<0.05). DTG/3TC genes compared to 3DR-INSTI revealed the up-regulation of the ATPase complex and down-regulation of the Oxidative stress pathway; meanwhile proteins revealed a down-regulation of the Biological oxidations with ALDH1A1, ACY1, ACY3, and ADH4 DEPs as leading markers. DTG/3TC compared to 3DR-non-INSTI genes showed an up-regulation of the TCA cycle with PC DEG as leading marker and down-regulation of the OXPHOS system with TMEM70 DEG as leading marker of the latest, while proteins revealed a down-regulation of the Biological oxidations enzymes and Oxidoreductase activity with ADH4, AKR7L1, DCK, and ADH1B DEPs as leading markers. 3DR-INSTI proteins compared 3DR-non-INSTI showed a down-regulation of the Fat digestion and Metabolism with CMTK1A, RBP2, FABP2, and DDC DEPs as leading markers. The direction of our findings was confirmed in the validation cohort.

Conclusion: This study suggests that DTG/3TC reduces mitochondrial and oxidative stress in PLHIV compared to 3DR containing NRTIs. We also showed the negative effects on fat metabolism of INSTI compared to non-INSTI based regimens.

816 Effect of Obesity on Response to Antiretroviral Therapy in SIV-Infected Rhesus Macaques
Kristin Sauter, Diana Takahashi, Melissa A. Kirigiti, Sarah R. Lindsley, Hannah Blomenkamp, Heather Hofmeister, Gabriela Webb, Oleg Varlamov, Jonah Sacha, Charles Roberts, Paul Kiewit
Oregon Health and Sciences University, Portland, OR, USA

Background: Modern antiretroviral therapy (ART) regimens are associated with increased risk of weight gain and overt metabolic disease. Adipokines play a role in adipose tissue dysfunction and are indicators of cardiometabolic disease risk. A reduced adiponectin:leptin ratio (ALR) is a predictive biomarker that correlates with a number of metabolic risk factors, as well as with markers of chronic inflammation. We employed the rhesus macaque model of SIV infection to determine if a modern ART regimen comprised of TDF, FTC, and DTG would elicit metabolic dysfunction with a corresponding decrease in the ALR, and if this effect was exacerbated by pre-existing obesity.

Methods: Lean, metabolically healthy (n=6) and western-style diet-induced obese (n=5) adult male macaques were infected i.v. with SIVmac239 and ART was initiated at 5 weeks post-infection and continued for 16 months. Baseline and longitudinal assessments of plasma, adipose tissue morphology, and systemic measures of metabolism were obtained.

Results: Unsurprisingly, the obese cohort exhibited a significantly lower ALR at baseline compared to the lean cohort (Obese 0.21±0.06 vs Lean 0.87±0.32, p=0.02). The lean cohort experienced a progressive decrease in ALR that was driven by a decrease in adiponectin, throughout the time course of chronic infection, ART initiation, and full suppression of viremia, that became significant at 56 weeks post-infection (PI) (Lean average 0.30±0.05). The change in ALR was inversely correlated to % change in body weight and fat mass, where animals in the lean group with the largest decrease in their ALR also had the greatest weight and fat mass gain. Additionally, the change in ALR in all animals was inversely correlated to % change in fasting insulin levels and HOMA-IR. The obese cohort also exhibited significantly elevated circulating C-reactive protein (CRP) and Lipopolysaccharide (LPS)-binding protein (LBP) at baseline compared...
Inflammatory Profile of B/F/TAF, DTG/ABC/3TC, and DTG+F/TAF Over 5 Years and Effects of Viral Blips

Nicholas Funderburg, Susie S. Huang, Calvin Cohen, Kate Ailstock, Jean Lee, Brenda Ng, Kirsten White, Jeff J. Wallin, Bryan Downie, Grace A. McComsey

The Ohio State University, Columbus, OH, USA; Gilead Sciences, Inc, Foster City, CA, USA; University Hospitals Cleveland Medical Center, Cleveland, OH, USA

Background: Elevated levels of inflammatory markers are linked to increased morbidity/mortality in people with HIV (PWH) and often remain elevated after suppression of HIV-1 replication below the limit of detection by antiretroviral therapy (ART). As new combinations of ART become available, an evaluation of their effects on biomarkers of inflammation and immune activation is crucial. We aimed to study the inflammatory profile of three ART regimens (B/F/TAF, DTG/ABC/3TC, and DTG+F/TAF) over a 5-year window and assess the impact of viral blips on selected inflammatory biomarkers.

Methods: We utilized cryopreserved samples from treatment-naive PWH enrolled in Phase 3 clinical studies investigating the efficacy and safety of B/F/TAF, DTG/ABC/3TC, and DTG+F/TAF over a 5-year window (GS-US-380-1489/1490). At wk144, participants were switched to open label B/F/TAF. We measured levels of interleukin-6 (IL-6), C-reactive protein (hsCRP), D-dimer, soluble CD14 (sCD14), and tumor necrosis factor-alpha (TNFα) by ELISA from select baseline, wk 24, 48, 144, and 240 samples (B/F/TAF, N=123; DTG/ABC/3TC, N=62; DTG+F/TAF, N=58). Samples from PWH who experienced a viral blip (n=44, defined as single VL>50c/ml) were also analyzed and paired with the most recent suppressed sample. Longitudinal biomarker changes were assessed using a constrained mixed effects linear regression model adjusting for covariates.

Results: Baseline demographics and selected laboratory characteristics were similar across studies. Significant decreases in D-dimer, sCD14, and TNFα were observed in all treatment arms, with no significant differences between arms at any timepoint. Similarly, biomarker levels also remained stable following ART-switch at week 144. No significant changes in hsCRP or IL-6 were observed in any arm at any timepoint. In the analysis of viral blips, a significant association was observed between sCD14 and increasing viral load (p=0.022); a similar trend was seen with D-dimer.

Conclusion: Viral suppression was associated with significantly reduced inflammation in treatment-naive PWH, with no significant differences in selected inflammatory biomarkers among the three ART regimens during the 144-week randomized period and each was sustained after the open label switch to B/F/TAF. Viral blips were associated with increases in some of the markers. The small number of available samples limited this study, thus the findings warrant additional investigation.

Impact of Raltegravir Intensification on the Gut Microbiota of People With Chronic HIV-1 Infection

Maria Casadellà, Alex Elizalde-Torrent, Francesc Català-Moll, Alessandra Borgognone, Mariona Parera, Marc Noquera-Julian, Roger Paredes

IrsiCaixa Institute for AIDS Research, Badalona, Spain

Background: Chronic human immunodeficiency virus 1 (HIV-1) infection is associated with gut microbiota alterations, including low gene richness and shifts in certain bacterial species, which have been linked to immune dysfunction. Residual HIV-1 replication might contribute to perpetuating such gut dysbiosis. We sought to explore if antiretroviral treatment (ART) intensification with raltegravir (RAL) had the ability to modify the gut microbiome composition and related immune parameters.

Methods: This was a prospective, double-blind, placebo-controlled, 2-arm randomized trial, where virologic subjects with HIV-1 under stable NNRTI- or PIr-based ART were randomized 2:1 to add RAL (1200 mg OD) or placebo to their ongoing ART, stratified by NNRTI vs PIr ART at study entry. We evaluated the longitudinal effect of RAL intensification on the gut microbiome by shotgun metagenomics as well as on soluble markers of immune activation and gut integrity (sCD14, DDimer, IFABP, IP10, and LBP) and cellular markers of immune activation and maturation (HLA-DR, CD83, exhaustion (PD-1) and senescence (CD57) at weeks 0, 12, 24 and 48. Non-parametric paired and unpaired tests and Linear Mixed Models (LMM) were used to analyse the data as needed.

Results: Fifty-seven subjects were included, 38 received RAL and 19 placebo. Microbial gene richness did not change in subjects receiving RAL but increased in those receiving Placebo (LMM p=0.009). There were no differences in beta-diversity between groups. In subjects receiving RAL, 3 Roseburia species (R. hominis, intestinalis and unilivorans) decreased over time, whereas Bifidobacterium longum and Paraprevotella xylanphila increased. Treatment intensification was associated with lower Streptococcus thermophilus abundance than placebo from week 12 onwards. All subjects remained aviremic throughout the study. RAL intensification was not associated with changes in CD4+, CD8+, sCD14, DDimer, IFABP, IP10, or LBP. In the placebo arm, we observed longitudinal increases in HLA+ effector memory and TEMRA CD8+ T-cells, as well as in CD83+ Naive CD8+ and CD57+ TEMRA CD4+ T-cells. Additionally, we noted decreases in CD57+ TEMRA CD8+ T-cells. No changes in cellular markers occurred in the RAL arm.

Conclusion: RAL intensification of PI/r or NNRTI-based regimens is associated with reduced immune activation and senescence in CD8+ T-cells, coupled with minor changes in the gut microbiome composition.
TAF) and abacavir (ABC), which are potent inhibitors of human telomerase activity, have been shown to negatively affect the BTL increase. We investigated the effect on BTL over 96 weeks after starting a dual therapy (DT) with delutegravir (DTG) plus lamivudine (3TC) vs a standard triple therapy (TT) with an anchor drug plus two NRTIs, one of which was TDF/TAF or ABC.

**Methods:** In this prospective longitudinal study we enrolled ART-naïve PLWH who started DT or TT, with no current AIDS event. We assessed BTL by monochrome multiplex qPCR (expressed as telomere to single-copy gene ratio, T/S) at the time of ART initiation (baseline, BL), virological success (VS) (achievement of HIV-RNA<50 copies/mL), and at weeks 48 (W48) and 96 (W96). We used an adjusted mixed model (GLM) to evaluate the effects of both the between- and within-subject factors. Linear regressions were performed to identify the variables associated with BL BTL and BTL changes over W96.

**Results:** From 2018-2022 we enrolled 71 participants: 41 in the TT and 30 in the DT group (Table1). Compared to TT, participants in DT were younger and with higher CD4 count. However, the two groups showed comparable BL HIV-RNA and HIV-DNA load, they similarly reached VS within 2 months and maintained viral suppression over the follow-up. At BL, the medians (IQR) of BTL were similar in the TT and DT groups: 9.3 (7.9-10.9) and 9.0 (7.8-10.0) for BTL, respectively, with a median ART duration of 5 years. A large number of sex differences were assessed in Cox proportional hazards models adjusted for VACS index and site. All p-values were adjusted for multiple comparisons by controlling for FDR using the Benjamini-Hochberg method.

**Conclusion:** In this setting, ART-naïve PLWH who initiated either DT or TT showed a similar evolution of BTL over time. They did not show any significant change in BTL after 1-year follow-up. However, a gain was observed at W96, suggesting a beneficial effect of ART, regardless of triple or dual regimen use.

### Table 1. Characteristics of participants at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>TT (N=41)</th>
<th>DT (N=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>47.3 (4.5)</td>
<td>44.0 (4.5)</td>
<td>0.068</td>
</tr>
<tr>
<td>CD4, cells/mm³</td>
<td>679 (342)</td>
<td>633 (304)</td>
<td>0.383</td>
</tr>
<tr>
<td>HIV-RNA, copies/mL</td>
<td>47.1 (15.6)</td>
<td>49.0 (13.7)</td>
<td>0.130</td>
</tr>
<tr>
<td>BTL, monochrome</td>
<td>9.0 (7.8-10.0)</td>
<td>9.0 (7.8-10.0)</td>
<td>0.130</td>
</tr>
</tbody>
</table>

**821 Age Modifies the Association Between Sex and the Plasma Inflammatory Proteome in Treated HIV**


**University of California San Francisco, San Francisco, CA, USA; Massachusetts General Hospital, Boston, MA, USA; University of California San Diego, La Jolla, CA, USA; University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; University of Alabama at Birmingham, Birmingham, AL, USA; University of Washington, Seattle, WA, USA.

**Background:** Among ART-suppressed people with HIV (PWH), women have higher levels of several plasma inflammatory markers than men, but the effect of sex on the larger plasma inflammatory proteome, and whether these differences are modified by age, remains unclear.

**Methods:** We analyzed 363 unique plasma proteins (Olink Inflammation Explore panel) within a randomly sampled sub-cohort of ART-suppressed (<400 copies HIV RNA/mL) CNICS participants. The relationship between natal sex and plasma proteins was assessed with linear regression models adjusted for age, natal sex, nadir CD4, site, race, MSM status, and clinical factors (smoking, IDU, HCV history, ASCVD risk score). Age-sex interaction terms were also assessed, stratifying age above and below the median age of 47, which in prior studies has approximated the average age of menopause in women with HIV. Mortality was separately assessed in Cox proportional hazards models adjusted for VACS index and site. All p-values were adjusted for multiple comparisons by controlling for the false discovery rate (FDR) using the Benjamini-Hochberg method.

**Results:** Of the 363 plasma proteins assessed, 103 died over a median follow-up of 9 years. Median age was 47; 162 (48%) were female. Median current and nadir CD4 count were 579 cells/mm³ and 245 cells/mm³, respectively, with a median ART duration of 5 years. A large number of sex differences in inflammatory markers were identified using significant evidence for an age-by-sex interaction for 157 of all 363 proteins assessed (43%) after FDR correction. In those ≤47 years (Panel A), women had lower levels of largely immunoregulatory proteins than men, of which were associated with higher mortality in the overall cohort. In contrast, in those >47 years, women had higher levels of predominantly inflammatory markers (36/42) than men, 26 of which were associated with higher mortality in the larger cohort (Panel B).

**Conclusion:** The impact of sex on the plasma inflammatory proteome is highly dependent on age among ART-suppressed PLWH, with women exhibiting more inflammation than men primarily at older ages. Whether menopause is responsible for unmasking these sex differences requires further study and is of high importance as many of these pathways are associated with increased mortality.

**822 Consequences of Low-Level Viremia by Sex Among People With HIV in the United States**

**Amalia Adregende, Cyra C. Mehta, Cecile D. Lahiri, Maria L. Alcaide, Kathryn Anastas, Todd T. Brown, Audrey L. Frenchi, Frank Palella, Michael Schneider, Phyllis Tien, Anandi N. Sethi, Lauren F. Collins, Emory University, Atlanta, GA, USA; University of Miami, Miami, FL, USA; Albert Einstein College of Medicine, Bronx, NY, USA; The Johns Hopkins University School of Medicine, Baltimore, MD, USA; Stoner Hope for the United States, Chicago, IL, USA; Northwestern University, Chicago, IL, USA; The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; University of California San Francisco, San Francisco, CA, USA.

**Background:** Low-level viremia (LLV) is common in people with HIV (PWHD) receiving antiretroviral therapy (ART) and has been associated with adverse outcomes including virologic failure (VF), drug resistance, and non-AIDS comorbidities (NACMs). As differences in these outcomes have been observed in men versus women with HIV, we investigated the effect of LLV on these outcomes by sex.

**Methods:** We included men enrolled in the Multicenter AIDS Cohort Study (MACS) and women in the Women’s Interagency HIV Study (WIHS) from 2003-2020 who reported ART use for ≥1 year with ≥2 consecutive HIV viral loads (VL) < 200 copies/mL. PWHD were then categorized using 4 consecutive VL results (baseline period) as: virologic suppression (VS; all VL below lower limit of assay detection), intermittent LLV (iLLV; VL>200 copies/mL, persistent LLV (pLLV; ≥2 consecutive detected VL 200-<2000 copies/mL) or VF (any VL ≥200 copies/mL). Outcomes were assessed from after baseline period through 5 years. Those with baseline VF were excluded. At first visit after baseline period, PWHD with multimorbidity (≥2 of NACM: hypertension, dyslipidemia, diabetes, cardiovascular disease, kidney disease) were excluded from that analysis.

**Results:** Of 2,395 PWHD, 57% were women, median age was 48 years, 53% were Black, 20% were Hispanic, median CD4 count was 616 cells/µL, and 89% reported ≥95% ART adherence. Over the baseline period (median 1.5 years), VS, iLLV, and pLLV occurred in 61%, 18%, and 6%, respectively. Among 1968 and 1123 PWHD included in each analysis, incident VF and multimorbidity occurred in 25% and 21%, respectively. Compared to PWHD with VS, the adjusted hazard ratio (aHR) for incident VF in women was 1.8 (95% CI 1.4,2.4) for iLLV and 2.4 (1.5,3.6) for pLLV, while in men was 1.4 (0.9,2.3) for iLLV and 3.1 (1.7,5.6) for pLLV (LLV*sex interaction p=0.04). Compared to PWHD with VS, the aHR for incident multimorbidity in women was 0.9 (0.6,1.3) for iLLV and 2.4 (1.5,3.6) for pLLV, while in men was 1.4 (0.9,2.4) for iLLV and 0.7 (0.2,1.9) for pLLV (LLV*sex interaction p=0.01).

**Conclusion:** In a diverse cohort of US PWHD, LLV was associated with an increased risk of developing VF, regardless of sex. There was a trend toward pLLV being associated with increased risk of multimorbidity in women but not in men. Further research is needed to mitigate the adverse consequences of LLV in men and women.
823 Short- and Long-Term Body Weight Gain Following Switch to Integrase Inhibitors Differ by Sex

Cecile D. Lahiri1, Cyra C. Mehta1, Dian Yang2, Jeffis Musonge-Effoe3, Julie B. Dandum4, Maria L. Alcâide5, Jordan E. Lake1, Leah H. Rubin5, Audrey L. French6, Jennifer Cocohoba7, Seble Kassaye8, Anjali Sharma9, Anandi N. Sheth10, Igho Ofotokun7, Jessica A. Alvarez1, Cyra C. Mehta1

1Emory University, Atlanta, GA, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 3University of Miami, Miami, FL, USA, 4University of Texas Health Science Center at Houston, Houston, TX, USA, 5The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 6Shogun Hospital of Cook County, Chicago, IL, USA, 7University of California San Francisco, San Francisco, CA, USA, 8Georgetown University, Washington, DC, USA, 9Monterrey Medical Center, Brownsville, TX, USA

Background: Integrase strand-transfer inhibitors (INSTIs) are associated with weight gain among persons with HIV (PWH), which may be more severe in women. We assessed differences in weight change by sex up to 6 years following switch to INSTIs.

Methods: We used data collected between 2007-2020 in virally-suppressed (<200 c/ml) INSTI-naive PWH on antiretroviral therapy (ART) for ≥2 years and persons without HIV (HIV-) enrolled in the MACS/WIHS Combined Cohort Study. We compared PWH who switched/added an INSTI to those who remained on non-INStI ART and to HIV-controls. Follow-up time was years since switch visit or comparable visit in controls. Weight change was the difference between post- and pre-switch visits. Linear regression mixed effect models assessed the effects of sex (assigned at birth), group, and time on absolute and percent (%) weight change, adjusted for age, race/ethnicity, socioeconomic status, and diabetes.

Results: 3466 participants contributed a mean 3.2 ±1.5 years of data, including 1940 women (411 INSTI, 711 Non-INStI, 818 HIV-) and 1526 men (223 INSTI, 412 Non-INStI, 691 HIV-). Compared to men, women were younger (47.2 vs 54.5 years), more likely to be non-Hispanic Black (65 vs 23%), had higher pre-switch BMI (31.5 vs 26.9 kg/m²), and higher prevalence of diabetes (19 vs 13%), respectively. Absolute weight gain in women versus men was +3.2 ±(±9.8) vs +1.6 ±(±6.1) kg in INSTI, +0.8 ±(±9.4) vs +1.0 ±(±6.6) kg in non-INStI, and +0.2 ±(±11.2) vs +0.7 ±(±2.2) kg in HIV- groups. Adjusted models, sex and group modified % weight change by time (sex*group*years interaction, p<0.0001). Men switching to INSTIs experienced greater % weight gain compared to non-INStI ART and HIV-controls up to 1 to 2 years post-switch: +2.42% (95% CI 1.41-3.42) vs +0.58% (-0.17-1.34) and +0.58% (0.02-1.14), respectively, with no differences between groups beyond 3 years. In contrast, women on INSTIs experienced higher % weight gain for up to 3–4 years post-switch compared to women on non-INStIs [+4.49% (95% CI 3.16-5.37) vs +2.08% (1.30-2.87)] and up to 5–6 years post-switch vs HIV- women [+5.16% (3.80-6.52) vs +0.02% (-1.24-1.28)], Figure.

Conclusion: Short and long-term body weight gain in PWH switching to INSTIs differed by sex, with women experiencing a greater amount and longer duration of weight gain relative to controls. Further research is needed to understand implications of weight gain on cardiometabolic disease and support sex-specific preventative and therapeutic intervention strategies.

824 Body Composition Changes Among DMPA and Non-Hormonal Users on TDF Based ART Switched to B/F/TAF

Flavia Kiwewa Matovu1, Martin Nabwana2, Esther Isingel3, Philippa Musoke4, Mary G. Fowler5, John Pettifor6, Todd T. Brown7, Mags Bekinska8, for the BONE: STAR Study Team

1Makere University–Johns Hopkins University Research Collaboration, Kampala, Uganda, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 3University of the Witwatersrand, Johannesburg, South Africa, 4Wits Reproductive Health and HIV Institute, Johannesburg, South Africa

Background: We previously demonstrated a higher bone mass among DMPA users on tenofovir alafenamide (TAF) compared with tenofovir disoproxil fumarate (TDF) containing ART. However, concerns about metabolic complications remain. We assessed the effect of switching women living with HIV (WLWH) from TDF/ lamivudine/ dolutegravir to Bitargravir /Emtricitabine /TAF (B/F/TAF; Biktarvy®) on body composition over a two-year period in the BONE: STAR study.

Methods: WLWH on TDF and DMPA-IM were randomized in a 1:1 ratio to either continue on a TDF based ART regimen (HIV+/DMPA+/TDF+) or switch to B/F/TAF (HIV+/DMPA+/TAF+). A third group of WLWH on TDF and using non-hormonal contraception were all offered B/F/TAF (HIV+/DMPA+/TAF+). Dual energy x-ray absorptiometry was used to measure lean mass, total, trunk, and extremity fat at enrollment and every 6 months for 2 years. Multivariable linear regression was used to assess differences in mean percent (%) change in fat and lean mass adjusting for age, race/ethnicity, socioeconomic status, and diabetes.

Results: A total of 346 WLWH were included in the analysis, with follow-up between December 2019 and August 2023. Median age was 31 years (interquartile range. 27.9 to 34.7 years). Both non-hormonal and DMPA groups who switched to B/F/TAF had significant increases in lean mean mass, trunk, and extremity fat post switch, p-value <0.001. There were no significant differences were in body composition parameters between women on B/F/TAF and DMPA or non-hormonal contraception, versus TDF users, p>0.173. Similarly, no differences were noted between women on B/F/TAF using DMPA versus non-hormonal contraception, p>0.125.

Conclusion: Significant increases were observed in fat and lean mass in both groups with no differences between DMPA users on TDF and DMPA or non-hormonal contraception switched to B/F/TAF. Longer term follow-up data beyond 2 years are needed to further understand the impact in WLWH of different ART options on fat gain and obesity-related conditions. The figure, table, or graphic for this abstract has been removed.

825 Sleep Apnea in People With and Without HIV in Primary Care

Jennifer O. Lam1, Craig E. Hou2, Stacey Alexeeff2, Joffi Musonge-Effoe3, Mary G. Fowler2, John Pettifor2, Todd T. Brown7, Mags Bekinska8, for the BONE: STAR Study Team

1Kaiser Permanente Northern California, Oakland, CA, USA, 2Kaiser Permanente Northern California, South San Francisco, CA, USA, 3Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA, 4University of California San Francisco, San Francisco, CA, USA

Background: Sleep apnea negatively impacts health and quality of life. A better understanding of potential differences in sleep apnea incidence and risk factors between people with HIV (PWH) and people without HIV (PWoH) could inform prevention and management strategies.

Methods: We conducted a study of adult (≥18-years old) members of Kaiser Permanente Northern California, an integrated U.S. healthcare system. PWH and PWoH with membership between July 2013 and December 2021 were matched 1:20 by age, sex, and race/ethnicity. Sleep apnea diagnoses were identified by
ICD codes in electronic health records. Using Poisson regression, incident sleep apnea was compared between PWH and PWoH, overall and with PWH stratified by HIV treatment status. Optimal HIV treatment was defined as being on ART (≥1 ART prescription fill), with undetectable HIV RNA (≤200 copies/ml), and without immunosuppression (CD4 count ≥500 cells/µl). Next, both HIV-specific and general (non-HIV-specific) risk factors were evaluated. All models included the following covariates: age, sex, race/ethnicity, body mass index, smoking status, substance use, depression, anxiety, cardiovascular disease, diabetes, cerebrovascular disease, cognitive impairment, and number of outpatient visits in the year before baseline. HIV-specific models also included ART use, HIV RNA level, and CD4 count.

Results: The study included 11,568 PWH and 225,097 PWoH (for PWH: mean baseline age 48 years, 90% men, 48% White, 20% Hispanic, 17% Black, 7% Asian, 8% Other/unknown race/ethnicity; 93% on ART). During follow-up, 820 PWH and 19,058 of PWoH were diagnosed with sleep apnea. Sleep apnea incidence was significantly lower in PWoH (vs. PWHO, adjusted incidence rate ratio (aIRR) = 0.90, 0.84-0.97). Notably, in analyses with PWH stratified by treatment status, lower incidence was observed in sub-optimally treated PWH (vs. PWHO, aIRR = 0.70, 0.70-0.89) but not in optimally treated PWH (vs. PWHO, aIRR = 0.97, 0.89-1.06). Among PWH, incidence was lowest in PWH with lower CD4 (vs. PWH with CD4 ≥500 cells/µl: CD4 200-499, aIRR = 0.89, 0.75-1.05; CD4 <200, aIRR = 0.58, 0.35-0.99) or with higher viral load (vs. PWH with HIV RNA <200 copies/ml: HIV RNA ≤10,000, aIRR = 0.59, 0.29-1.19). The associations of general risk factors with sleep apnea were similar by HIV status (data not shown).

Conclusion: Sleep apnea incidence is independent of HIV status. Lower overall incidence of sleep apnea among PWH may reflect under-diagnosis in PWH with untreated or uncontrolled HIV infection.

827 Protease Activity in the Lung in HIV-Associated Obstructive Lung Disease (OLD)

Chris Wendt1, Sarah Samorednitsky1, Monica Kruk1, Eric Lock2, Alison Morris3, Janice Leung1, Danielle Weise1, Laurine Parker1, Pratik Jagtap1, Timothy Griffin1, Ken Kunisaki1
1University of Minnesota, Minneapolis, MN, USA, 2University of Pittsburgh, Pittsburgh, PA, USA, 3University of British Columbia, Vancouver, Canada

Background: Obstructive lung disease (OLD) is increasingly common among persons with HIV (PWH). The role of proteases in HIV associated OLD is unknown.

Methods: We performed mass spectrometry (MS) analysis on endogenous peptides and tandem mass tagging for protein analysis on bronchoalveolar lavage fluid (BALF) samples from PWH with OLD (n=25) and without OLD (n=26) matched on smoking status and ART. We combined untargeted MS and targeted Somascan aptamer-based proteomic approaches to quantify individual proteases and their correlation with lung function. We mapped endogenous peptides to their native proteins that were subjected to protease activity and the accompanying proteases responsible for the peptide cleavages. We used t-tests to compare average FEV1pp between samples in which each cleaved protein was detected versus absent. We accounted for multiple comparisons using a false discovery rate (FDR) adjustment. Using the MEROPS database, we identified candidate proteases for peptide generation by quantifying their affinity to binding sites via z-scores. We assigned proteases as likely responsible for cleavage by the z-scores for each peptide.

Results: We identified 27 proteases that correlated with lung function. Proteases associated with low FEV1pp included myeloblastin, kallikrein, cathepsins, metalloproteinases, caspase and neutrophil elastase. Proteases associated with high FEV1pp included carboxypeptidase M, prothrombin, urokinase and gastrin. MS analysis of endogenous peptides identified 1402 proteins that mapped to these peptides, 28 of which were observed in individuals with significantly (FDR <= 0.1) lower average FEV1pp. The top five protease targeted proteins included: alpha-enolase, gelsolin, histone H4, tubulin beta-4 chain and histone H2B type 2-F. Pathway analysis revealed these proteins were associated with gene regulation and included SUMOylation, methylate and demethylation histones, and nucleosomes. The top five proteases demonstrating activity included: neutrophil elastase, granzyme M, cathepsin D, protease 3, and cathepsin E (Fig. 1).}

Conclusion: We identified the role of proteases in HIV-associated OLD. Proteases involved in gene regulation are susceptible to these proteases as potential therapeutic targets.

826 Outcome of a Multidimensional Intervention for Insomnia in a Cohort of People Living With HIV

Maria Mazzitelli1, Mattia Trunfio1, Lolita Sasset2, Davide Leoni2, Vincenzo Scaglione2, Mauro Marini2, Gianluca Garparini2, Angela Favaro3, Annamaria Cattelan1
1Azienda Ospedaliera di Padova, Padua, Italy, 2University of California San Diego, La Jolla, CA, USA, 3University of Padova, Padova, Italy

Background: No studies specifically assessed interventions for improving sleep quality (SQ) in people with HIV (PWH). We introduced a multidimensional program for SQ in the routine management at our outpatient clinic and assessed its impact on PWH suffering from insomnia.

Methods: Interventional study in adult PWH with subthreshold (≥8), moderate (≥15), or severe (≥22) insomnia at the Insomnia Severity Index (ISI). Participants received sleep hygiene counseling and underwent tailored interventions as shown in the study flow. Based on compliance with prescribed interventions, participants were classified as fully, partially (at least 1 intervention attended when more than one was prescribed), or non-adherent (FA-PA-NQ). SQ, depression (PHQ9), and well-being were assessed at baseline and after 6 months from the beginning of any interventions. The impact of interventions on insomnia was shown in the study flow. Based on compliance with prescribed interventions.

Results: Among 730 PWH screened, 277 had altered ISI score, and 175 with ≥1 ART prescription fill), with undetectable HIV RNA (<200 copies/ml), and without immunosuppression (CD4 count ≥500 cells/µl). Next, both HIV-specific and general (non-HIV-specific) risk factors were evaluated. All models included the following covariates: age, sex, race/ethnicity, body mass index, smoking status, substance use, depression, anxiety, cardiovascular disease, diabetes, cerebrovascular disease, cognitive impairment, and number of outpatient visits in the year before baseline. HIV-specific models also included ART use, HIV RNA level, and CD4 count.

Results: The study included 11,568 PWH and 225,097 PWoH (for PWH: mean baseline age 48 years, 90% men, 48% White, 20% Hispanic, 17% Black, 7% Asian, 8% Other/unknown race/ethnicity; 93% on ART). During follow-up, 820 PWH and 19,058 of PWoH were diagnosed with sleep apnea. Sleep apnea incidence was significantly lower in PWoH (vs. PWHO, adjusted incidence rate ratio (aIRR) = 0.90, 0.84-0.97). Notably, in analyses with PWH stratified by treatment status, lower incidence was observed in sub-optimally treated PWH (vs. PWHO, aIRR = 0.70, 0.70-0.89) but not in optimally treated PWH (vs. PWHO, aIRR = 0.97, 0.89-1.06). Among PWH, incidence was lowest in PWH with lower CD4 (vs. PWH with CD4 ≥500 cells/µl: CD4 200-499, aIRR = 0.89, 0.75-1.05; CD4 <200, aIRR = 0.58, 0.35-0.99) or with higher viral load (vs. PWH with HIV RNA <200 copies/ml: HIV RNA ≤10,000, aIRR = 0.59, 0.29-1.19). The associations of general risk factors with sleep apnea were similar by HIV status (data not shown).

Conclusion: Sleep apnea incidence is independent of HIV status. Lower overall incidence of sleep apnea among PWH may reflect under-diagnosis in PWH with untreated or uncontrolled HIV infection.

Methods: We performed mass spectrometry (MS) analysis on endogenous peptides and tandem mass tagging for protein analysis on bronchoalveolar lavage fluid (BALF) samples from PWH with OLD (n=25) and without OLD (n=26) matched on smoking status and ART. We combined untargeted MS and targeted Somascan aptamer-based proteomic approaches to quantify individual proteases and their correlation with lung function. We mapped endogenous peptides to their native proteins that were subjected to protease activity and the accompanying proteases responsible for the peptide cleavages. We used t-tests to compare average FEV1pp between samples in which each cleaved protein was detected versus absent. We accounted for multiple comparisons using a false discovery rate (FDR) adjustment. Using the MEROPS database, we identified candidate proteases for peptide generation by quantifying their affinity to binding sites via z-scores. We assigned proteases as likely responsible for cleavage by the z-scores for each peptide.

Results: We identified 27 proteases that correlated with lung function. Proteases associated with low FEV1pp included myeloblastin, kallikrein, cathepsins, metalloproteinases, caspase and neutrophil elastase. Proteases associated with high FEV1pp included carboxypeptidase M, prothrombin, urokinase and gastrin. MS analysis of endogenous peptides identified 1402 proteins that mapped to these peptides, 28 of which were observed in individuals with significantly (FDR <= 0.1) lower average FEV1pp. The top five protease targeted proteins included: alpha-enolase, gelsolin, histone H4, tubulin beta-4 chain and histone H2B type 2-F. Pathway analysis revealed these proteins were associated with gene regulation and included SUMOylation, methylate and demethylate histones, and nucleosomes. The top five proteases demonstrating activity included: neutrophil elastase, granzyme M, cathepsin D, protease 3, and cathepsin E (Fig. 1).
828 Safety of Tenofovir Alafenamide in Individuals With a History of Proximal Renal Tubulopathy on TDF
Lucy Campbell1, Birgit Barbin1, Ben Cromarty2, Lisa Hamzah2, Margaret Johnson6, Deborah Williams6, Alan Winston5, Frank A. Post1, for the FANTA Trial Team
King’s College London, London, United Kingdom, 1UK Community Advisory Board, London, United Kingdom, 2St George’s University of London, London, United Kingdom, 3Royal Free Hospital, London, United Kingdom, 4Brighton and Sussex University Hospitals NHS Trust, Brighton, United Kingdom, 5Imperial College London, London, United Kingdom, 6King’s College Hospital NHS Foundation Trust, London, United Kingdom

Background: Proximal renal tubulopathy (PRT, Fanconi syndrome) is an important but uncommon complication of tenofovir disoproxil fumarate (TDF). There are few long-term safety data for tenofovir alafenamide (TAF) in this population. We evaluated the safety of TAF in individuals who experienced treatment-limiting PRT while receiving TDF, and here report the five-year outcomes.

Methods: Participants with HIV, a history of TDF-associated PRT, an estimated glomerular filtration rate >30 mL/min/1.73 m², HIV RNA <200 copies/mL, and who were no longer receiving TDF and naïve to TAF were switched to a TAF-based antiretroviral therapy (ART) regimen and followed up annually for five years. The primary outcome was recurrent PRT. Secondary outcomes were changes in kidney biomarkers, alkaline phosphatase, and bone mineral density (BMD). Data were analysed using multi-level mixed effects linear regression models. The trial was registered under EuDraCT 2016-003345-29.

Results: Of the 28 study participants (median age 55 [IQR 51, 60] years, 96% male, 86% white ethnicity) who agreed to continue follow up beyond week 96, 26 remained on TAF at year 5. Two participants (7%) discontinued TAF (treatment simplification, pre-emptive switch during critical care unit admission for COVID-19), 2 participants (7%) experienced transient HIV viraemia (200-1000 copies/mL) while all others remained virally suppressed; none experienced recurrent PRT during 134 person-years of follow up. Participants experienced small declines in BMD at the total hip; there were no significant changes in estimated glomerular filtration rate (eGFR-creatinine), albuminuria, proteinuria, fractional excretion of phosphate, alkaline phosphatase, or BMD at the lumbar spine.

Conclusion: In individuals with a history of PRT on TDF, cumulative exposure to TAF-based ART for five years was not associated with recurrent PRT or adverse effects on renal function or BMD. These data suggest that TAF is a safe treatment option for this vulnerable population.

830 Biomarkers Influence Kidney Function Estimates More So Than Race Among Persons With HIV
Peggy-Ita A. Obeng-Nyarko1, Amanda B. Spence2, Richard Terani3, Christopher A. Lefford4, Bruce Luxon5, Joseph Timpane6, Princy Kumar5, Jason Umans7, Sebile Kassaye8, Geoffrey S. Kondracki9, Jonathan J. Diouf10, Lisa Hamzah11, Ben Cromarty1, Birgit Barbin1, Ben Cromarty2, Lisa Hamzah2, Margaret Johnson6, Deborah Williams6, Alan Winston5, Frank A. Post1, for the FANTA Trial Team
King’s College London, London, United Kingdom, 1UK Community Advisory Board, London, United Kingdom, 2St George’s University of London, London, United Kingdom, 3Royal Free Hospital, London, United Kingdom, 4Brighton and Sussex University Hospitals NHS Trust, Brighton, United Kingdom, 5Imperial College London, London, United Kingdom, 6King’s College Hospital NHS Foundation Trust, London, United Kingdom

Background: Kidney function estimation equations were revised in 2021 to exclude race, refuting earlier assumptions that creatinine (Cr), a by-product of muscle metabolism, consistently differs by race. Cystatin C (CysC) is a biomarker produced by nucleated cells and can be used in conjunction with creatinine to estimate kidney function. We sought to understand the effects of different estimating equations and biomarkers on Chronic Kidney Disease (CKD) stage estimates among persons with HIV (PWH), for whom CKD is an important comorbidity.

Methods: CysC and Cr was measured in this cross-sectional single site U.S. clinic-based study from 2014-2016. The 2009 CKD-EPI-Cr and 2012 CKD-EPI-Cr-CysC (which include race), and 2021 CKD-EPI-Cr and 2021 CKD-EPI-Cr-CysC (which exclude race) estimating equations were applied to categorize CKD stage, and agreement was assessed using proportions of regressions. Proportion analyses evaluated factors associated with CKD stage, and the Breslow-Day test evaluated whether race served as an effect modifier.

Results: Among 306 PWH, the median age was 48.2 years, 86 (28.1%) were female, 185 (60.5%) were Black, 91 (29.7%) Caucasian, 13 (4.3%) Latinx, and 46 (15%) had HIV co-infection. The median CD4+ count was 659/mm³, 97.7% were on ART, and 74.5% had HIV VL < 20 c/ml. Using the 2009 and 2012 equations (including race), more individuals were categorized as having normal kidney function (Stage I) with inclusion of CysC than Cr alone (73.2% vs 54.6%, p < 0.00001); fewer individuals were classified in CKD stages III-V using CysC than Cr alone, but this did not meet statistical significance (8.2% vs 11.8%, p = 0.14). Using 2021 equations (excluding race) a larger proportion were classified as normal kidney function with inclusion of CysC than Cr alone (73.8% vs 49.3%, p = 0.00001); fewer were categorized as CKD III-V with inclusion of CysC than Cr alone (8.1% vs 13.1%, p = 0.026). Multivariate linear regression identified age, the interaction between tobacco use and absolute CD4+ count, and the interaction between HIV co-infection and nadir CD4+ count as factors associated with kidney function. Race was not an effect modifier based on our analyses.

Conclusions: Among PWH, CysC shifted estimates of kidney function towards normal and resulted in shifts in kidney function categorization much more so than the small race effect. As some antiretrovirals raise creatinine without affecting renal function, CysC remains an important but under-utilized biomarker to confirm diminished kidney function.
Markers of Macrophage Activation and Kidney Function in People With and Without HIV

Molly Fisher1, David B. Hanna2, Anjali Sharma3, Todd T. Brown4, Michelle Floris-Moore5, Frank Palaia6, Jordan E. Lake7, Seble Kassaye8, Susan L. Koletar9, Eric Seaberg10, Igbo Ofotokun11, Rubin Hickman12, Alan Lanyado13, Robert Kaplan14, Michael Ross15

1Montefiore Medical Center, Bronx, NY, USA, 2Makerere University–Johns Hopkins University Research Collaboration, Kampala, Uganda, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 4Northern Western University, Chicago, IL, USA, 5University of Texas at Houston, Houston, TX, USA, 6Georgetown University, Washington, DC, USA, 7The Ohio State University, Columbus, OH, USA, 8The Johns Hopkins University, Baltimore, MD, USA, 9Emory University, Atlanta, GA, USA, 10University of Mississippi Medical Center, Jackson, MS, USA, 11Rush University, Chicago, IL, USA, 12Albert Einstein College of Medicine, Bronx, NY, USA

Background: HIV infects and activates macrophages, which produce important mediators of inflammation that drive age-related comorbidities. Soluble macrophage activation markers are higher in people with HIV (PWH), even with viral suppression, and have been associated with subclinical cardiovascular disease. Given the close relationship between cardiovascular and kidney disease, we hypothesized these markers would be associated with lower kidney function among PWH.

Methods: We performed a cross-sectional study nested within two prospective U.S. multicenter cohorts each of persons with and without HIV; the Women's Interagency HIV Study (WIHS) and for men the Multicenter AIDS Cohort Study (MACS). Serum levels of the macrophage activation markers soluble (s)CD163, sCD14, galectin-3 (Gal-3) and Gal-3 binding protein (Gal-3BP) were measured in 786 (73% PWH) women and 498 (65% PWH) men. Markers were log and Z-score transformed. Multivariable linear regression determined associations of these markers with estimated glomerular filtration rate (eGFR), adjusting for potential confounders including study site, sociodemographics, diabetes, hypertension, hepatitis C, HIV serostatus and tenofur disoproxil fumarate use.

Results: Among 1284 participants, 786 (61%) were women, 902 (70%) were PWH, of whom 486 (54%) were virally suppressed (<50 cp/mL). Median age was lower among women compared to men (40 vs 49 years). sCD14, sCD163 and Gal-3BP levels were significantly higher among PWH versus without HIV (p<0.05). Median eGFR was higher among PWH versus without HIV (95.3 vs 93.6 ml/min in men and 92.7 vs 85.8 ml/min in men). After confounder adjustment, Gal-3 and sCD14 levels were negatively associated with eGFR. Each standard deviation (SD) increase in Gal-3 was associated with a 1.71 ml/min lower GFR (95% confidence interval: -2.66, -0.84; p=0.0004) and each SD increase in sCD14 was associated with a 1.87 ml/min lower GFR (-2.92, -0.82; p=0.0005). These associations were more pronounced among PWH (p for interaction 0.08 and 0.06, respectively). A weak positive association existed between sCD163, and eGFR and no association was observed between Gal-3BP and eGFR.

Conclusion: The macrophage markers Gal-3 and sCD14 may have a role in subclinical kidney injury among PWH. Future work will examine macrophage associations with longitudinal kidney outcomes to identify whether they may play a causative role and/or have predictive value for kidney disease risk.

Table 1. Distribution of kidney function estimates based on estimating equations

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<td>End-stage</td>
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Background: HIV-associated nephropathy (HIVAN), but people living with HIV (PLWH) remain at high risk for later developing chronic kidney disease (CKD), with a poorly defined etiology. HIV acquisition is associated with reduced plasma anti-α4β7 integrin (α4β7) and increased pro-fibrotic galectin (Gal)-3. Clinical trials investigating α4β7 receptor agonists and Gal-3 antagonists to ameliorate HIVAN/CKD were completed but discontinued after phase 2, necessitating new strategies to restore kidney function. FDA-approved vedolizumab (anti-α4β7) is well-tolerated for inflammatory bowel diseases. Here, we query whether anti-α4β7 can improve renal function in a rhesus macaque (RM) infection model.

Methods: Nine RMs were CD8-depleted and inoculated with SIVmac251. Daily cART was begun at week two, along with infusions (anti-α4β7 mAb: n=5; IgG controls: n=4) every three weeks until week 23. CART was discontinued (ATI) at week 14. Longitudinal plasma measurements were performed for serum chemistry, retinoids determined by HPLC, and Gal-3 by Luminex. α4β7 metabolism and podocyte differentiation gene expression were determined by qPCR in kidney samples collected at necropsy and compared to uninfected RMs (n=4).

Results: Anti-α4β7 treated RMs had lower measures of urea nitrogen (BUN) and BUN:Creatinine ratio than IgG RMs following therapy and ATI (P=0.0139; P=0.0250), despite comparable measures at baseline. Acute infection was associated with reduced (~35% less) α4β7Rα in both groups (P=0.0046; P=0.0014). While α4β7Rα remained low in IgG RMs, it increased in anti-α4β7 RMs during CART (P=0.0012) and ATI (P=0.0004), resulting in differences between groups (P=0.0101; P=0.037). α4β7Rα receptor retinyl ester (RE) was higher in IgG RMs (P=0.0139) than anti-α4β7 RMs after ATI. Relative was positively correlated with BUN:Creatinine (P=0.0125). Anti-α4β7 RMs had greater α4β7 synthesis (ALDH1A1, ALDH1A3) while IgG RMs had higher α4β7 catabolism (CYP26A1) gene expression than uninfected RMs. IgG (but not anti-α4β7) RMs also had lower SYNOPO and BAIIIRES expression. IgG RMs had lower Gal-3 than anti-α4β7 RMs (P=0.0044 after ATI). Gal-3 levels associated negatively with SYNOPO expression (P=0.0299) and positively with RE (P=0.0275) and BUN:Creatinine (P=0.044).

Conclusion: Our data provide a rationale for repurposing vedolizumab for HIVAN/CKD to restore retinoid signaling, podocyte differentiation, and glomerular filtration thereby improving quality of life in PLWH.

Depressive Symptom Burden Predicts Diabetes Mellitus Control Among People Living With HIV

Aima A. Ahonkhai1, Alhuma Bisan2, Paridhi Kanadive3, Greer Buckholder4, Richard D. Moore5, Barbara M. Griphove6, Edward Cachay7, Jennifer P. Jain7, Kenneth H. Mayer5, Deana Agil8, April Pettit9, Mani Kitahata10, Heidi M. Crane11, Bryan E. Shepherd12, Jessica L. Castillo13

1Vanderbilt University, Nashville, TN, USA, 2University of Alabama at Birmingham, Birmingham, AL, USA, 3The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4University Hospitals Cleveland Medical Center, Cleveland, OH, USA, 5University of California San Diego, La Jolla, CA, USA, 6University of California San Francisco, San Francisco, CA, USA, 7Fenway Health, Boston, MA, USA, 8University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 9Vanderbilt University, Nashville, Tennessee, 10University of Washington, Seattle, WA, USA

Background: Depression is common among people with HIV (PWH) and increases the risk of non-communicable diseases such as diabetes mellitus (DM). Depressive symptoms are associated with poorer medication adherence and adverse HIV outcomes, but less is known about how depression affects DM outcomes. We examined the association between depressive symptoms and DM control among PWH and DM.

Methods: We examined PWH and DM (defined by HbA1c ≥6.5% or receipt of DM specific medication, or DM related medication and DM diagnosis) cared for at 8 US clinical sites in the CFAR Network of Integrated Clinical Systems (CNICS) cohort from 2005-2023 and who had repeated measures of patient-reported depressive symptoms using the Patient Health Questionnaire (PHQ-9). We observed PWH from the date of first HbA1c with a PHQ9 score within 15 months prior and assessed diabetes outcomes using categorical (cs/>7.0%) and continuous HbA1c values. We examined the association between HbA1c and recent depressive symptoms (PHQ9 measured within the 15 months prior to or at the date of HbA1c) using multilevel longitudinal models whereby PWH could
834 Effectiveness of Electronic Screening for Substance Use, Depression, and Anxiety in HIV Primary Care

Michael J. Silverberg1, Tony Levine1, Varada Saravar1, Alexandra Lea1, Amy S. Leibowitz1, Michael A. Horberg2, Charles B. Hare3, Mitchell N. Levy1, Jason A. Flamm4, Derek D. Satre5, Kaiser Permanente Northern California, Oakland, CA, USA, Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA, Kaiser Permanente San Francisco Medical Center, San Francisco, CA, USA, Kaiser Permanente Oakland Medical Center, Oakland, CA, USA, Kaiser Permanente Sacramento Medical Center, Sacramento, CA, USA, University of California San Francisco, San Francisco, CA, USA

Background: Substance use (SU), depression, and anxiety are common in persons with HIV (PWH) yet are often not diagnosed or treated.

Methods: The Promoting Access to Care Engagement (PACE) study is a stepped wedge trial to evaluate the effectiveness of electronic SU and mental health (MH) screening to increase treatment among PWH. PACE was conducted from October 2018-July 2020 in 3 large HIV primary care clinics in Kaiser Permanente Northern California, serving 5115 PWH. The intervention involved an electronic survey (via patient portal or clinic tablets) consisting of: Tobacco, Alcohol, Prescription medication and other Substance use (TAPS); Patient Health Questionnaire-9 (PHQ-9); and Generalized Anxiety Disorder-2 (GAD-2), with results visible in the electronic health record. Each clinic had a 2-year pre-interventional comparison period. The study included PWH with incident SU and/or MH first identified (clinically or study survey). The outcome was % treated by interventional comparison period. The study included PWH with incident SU and/or MH first identified (clinically or study survey). The outcome was % treated by interventional comparison period. We compared % treated in 3 groups: 6 months, defined by: medications (antidepressant, antianxiety, SU), specialty care, or behavioral health specialist visits. We compared % treated in 3 groups: (1) pre-intervention (ref), (2) post-intervention with SU or MH first identified (2) clinically (3) by study survey. Adjusted hazard ratios (HR) from Cox models (variables in Table footnote).

Results: 1,988 PWH had evidence of SU problems; N=1285 pre-intervention; N=703 post-intervention (49% by survey). As shown in the Table, % treated was similar comparing PWH in pre- and post-intervention periods identified clinically (33% vs. 33%), but lower in PWH identified by survey in post-intervention period (26%). 2119 PWH had evidence of MH problems: N=1099 pre-intervention; N=1020 post-intervention (73% by survey). % treated was lower, compared with pre-intervention (83%), for PWH in post-intervention period identified clinically (73%), and by survey (65%). Results were similar in adjusted model (Table). Excluding follow-up during COVID-19 (after Mar 2020) did not impact findings (data not shown).

Conclusion: Systematic screening using validated survey tools in HIV primary care identified 49% SU and 73% MH concerns previously unrecognized by providers. The reduced % treated for these PWH suggest systematic screening may identify lower-severity groups overall.

![Figure 1. Depression Outcomes between People with HIV and Depression in a Group Therapy Intervention Group and a Control Group with Enhanced Usual Care](image-url)

835 Group Therapy Reduces Depression Among People Newly-Enrolled in HIV Care in Kampala, Uganda

Sarah Lofgren1, Vanessa Akinyange2, Angela Arinda1, Raymond Sebuliba1, David J. Bond1, David R. Boulware1, Noeline Nakasajja2, University of Minnesota, Minneapolis, MN, USA, Makerere University, Kampala, Uganda, The Johns Hopkins University, Baltimore, MD, USA

Background: Depression in people living with HIV (PWH) is associated with reduced medication adherence, viral suppression, and retention-in-care. In low-resourced settings, depression interventions are needed for PWH to improve HIV outcomes.

Methods: This study was conducted at a large, public HIV clinic. We enrolled PWH who started HIV medications within the last 3 months and had depression. They were randomized 1:1 into an 8-week group therapy intervention or enhanced usual care. The group therapy was held once a week, facilitated by adherence counselors at the subjects’ HIV clinic in groups of 6-10 individuals divided by biological sex. Depression was measured with the Patient Health Questionnaire, PHQ, with mild and moderate depression cutoffs at 5 and 10. Basic statistics were calculated. Groups were compared using the t-test and chi-square.

Results: Between February 2021 and March 2023, we enrolled 135 PWH into our study. The average age was 32 years; The group was 64% female. Of 46 PWH with data on chart review, the average CD4 cells/μL count was 369. Baseline PHQ-9 were similar between the intervention and control groups at median (IQR) 7.5 (6.5-9.5) and 7.0 (5.0-8.0), (p=0.36). Overall, 37% of those in the group therapy intervention group and 38% in the control group had a PHQ-9 score of >10 signifying moderate to severe depression. Of those enrolled in group therapy, 98% completed all 8 sessions. Of the 40 completing a satisfaction survey, 93% felt group therapy was acceptable. At three months, fewer subjects in group therapy had PHQ-9 score median (IQR) 1.5 (1.0-3.0) versus 4.0 (3.0-6.0), p<0.003; PHQ-9 score of >5, 11% versus 34%, p=0.005. By three months, only 1 of the 109 subjects overall had a PHQ-9 score of >10. By six months, the PHQ-9 had normalized in most with median (IQR) 0.0 (0.0-2.0) from intervention group versus 2.0 (1.0-3.0) from control group, (p=0.09). Only 2% of those in the group therapy group and 7% in the control group had mild depression (p=0.35). No subjects had a PHQ-9 score of >10. There were two Covid-19 lockdowns during this study, which impacted follow-up rates as people moved to the countryside and back to different parts of the city.

Conclusion: An 8-week group therapy depression intervention was successful in relieving depression in PWH newly enrolled in clinic by three months compared to control. By 6-month follow-up, depression had resolved in nearly all subjects. Group therapy can be adapted to different settings and is effective in improving mood in PWH.
Biomarkers of Microbial Translocation and Inflammation Associated With Frailty Among People With HIV
Stephanie A. Ruderman, Peter W. Hunt, Amanda Willig, Michael S. Saag, Sonia Napravnik, Edward Cachay, Laura Bamford, Lydia N. Drumright, Lynside S. Mixson, Bridget Whitney, Robin M. Nance, Mari Kitahata, Heidi M. Crane, Joseph A. Delaney, Andrew Hahn
University of Washington, Seattle, WA, USA, 2University of California San Francisco, San Francisco, CA, USA, 3University of Alabama at Birmingham, Birmingham, AL, USA, 4University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 5University of California San Diego, San Diego, CA, USA, 6University of California San Diego, La Jolla, CA, USA

Background: Frailty is observed at high rates among people with HIV (PWH) and occurs at younger ages compared to the general population. This higher burden of frailty is often attributed to chronic inflammation and subsequent immune exhaustion, which is increasingly being targeted as a means for mitigating frailty progression and impacts. We assessed how a panel of biomarkers of inflammation, measured in a case cohort sub-study of PWH in clinical care, is associated with frailty among PWH.

Methods: A panel of 13 plasma inflammatory biomarkers was collected at a single timepoint from a subset of virally suppressed PWH in the Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) cohort between 2010-2018. CNICS also collects and harmonizes data on demographic information, laboratory values, diagnoses, medications, and patient reported outcomes (PROs). Frailty over time was measured with a validated phenotype of 4 components (inactivity, immobility, fatigue, and unintentional weight loss) from the PRO assessment from biomarker date through July 2022. We considered frailty scores ranging from 0-4, with one point per endorsed component. We used linear mixed models to estimate longitudinal associations between standard deviation-scaled biomarkers and frailty score, with adjustment for demographic characteristics, clinical factors and comorbidities, and coinfections.

Results: Among 273 PWH, most were male (91%), average age at baseline was 45 years, 45% were non-Hispanic White while 35% were non-Hispanic Black, and average follow-up time was 6.2 years. Several inflammatory biomarkers were associated with higher frailty scores, including those linked to microbial translocation (soluble CD14 [sCD14], lipopolysaccharide binding protein [LBP], kynurenine-to-tryptophan [KT] ratio) and systemic inflammation (C-reactive protein [CRP] and soluble tumor necrosis factor receptor 2 [sTNFR2]) (Figure 1). For example, a standard deviation higher KT ratio was associated with a 0.14-point higher frailty score (95% CI: 0.04-0.25), CRP was associated with a 0.25-point higher frailty score (95% CI: 0.16-0.34) and sCD14 was associated with a 0.20-point higher frailty score (95% CI: 0.09-0.30) over follow-up.

Conclusion: Higher levels of biomarkers linked to microbial translocation and systemic inflammation are associated with higher frailty scores over time in a cohort of virally suppressed PWH, highlighting these pathways as potential interventional targets for preventing or reducing frailty in treated PWH.

Excess Inflammation Associated With AIDS and Non-AIDS Complications in Adults on ART
Kanai Singh, Shweta Sharma, Birgit Grund, Alejandro Arenas-Pinto, Inakelu Erubu, Edward Gardner, Win Min Han, Jose Hidalgo, Jennifer Hoyt, Jakob Malin, Daniel Murray, Siegfried Schwarz, Alan Winston, Jason Baker
The INSIGHT START Group
1National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, 2University of Minnesota, Minneapolis, MN, USA, 3University College London, London, United Kingdom, 4Institute of Human Virology Nigeria, Abuja, Nigeria, 5Denver Health Medical Center, Denver, CO, USA, 6HIV-Net, Bangkok, Thailand, 7Via Libre, Lima, Peru, 8The Alfred Hospital, Melbourne, VIC, Australia, 9Cologne University Hospital, Cologne, Germany, 10Hospitalet, Copenhagen, Denmark, 11European AIDS Treatment Group, Brussels, Belgium, 12Imperial College London, London, United Kingdom, 13Hennepin Healthcare Research Institute, Minneapolis, MN, USA

Background: In START, immediate ART initiation lowered inflammation and risk for clinical events. It is unclear to what degree prolonged inflammation contributes to clinical risk after ART is initiated. We quantified the excess inflammation associated with ART deferral in START, and investigated its influence on clinical event risk during viral suppression.

Methods: The START trial (2009-2021) randomized participants (pts) with CD4>500 cells/µL to immediate or deferred (when CD4 <350 cells/µL) ART. In 2015, trial results were unblinded and all pts were advised to initiate ART. In a substudy, plasma IL-6 and D-dimer (biomarkers) levels were measured at baseline, month 8 and annually. Longitudinal averages of biomarker levels from study entry through 2015 were compared between treatment arms using rank-sum tests. Kaplan-Meier curves for time to a composite outcome (AIDS, serious non-AIDS [SNA], or death) that occurred between 2016-2021 were estimated for the upper quartile (Q4) versus the lower 3 quartiles (Q1-Q3) of average longitudinal biomarkers through 2015. Associations between average biomarker levels through 2015 and the composite were estimated using Cox models adjusted for treatment arm.

Results: The analysis included 2114 pts. Through 2015 (median of 3.2 yrs), the deferred compared to the immediate arm had higher longitudinal biomarker levels: median IL-6 1.7 vs 1.5 pg/mL, D-dimer 0.33 vs 0.27 mg/mL (both p<0.001). By 01Jan2016, 87% of pts in the deferred and 99% in the immediate arm started ART; median time to ART start in the deferred arm was 2.5 years. There were 124 AIDS, SNA, or death events from 2016-2021; 0.92 and 1.27 per 100PY in immediate and deferred arms, respectively; HR(imm/def)=0.7 (95% CI 0.5-1.0; p=0.07). Higher longitudinal IL-6 & D-dimer levels through 2015 were associated with higher risk of the composite during 2016-2021 in both arms (Figure); HR(Q4 vs Q1-Q3)=2.3 (95% CI 1.6-3.2) for IL-6 and 2.1 (95% CI 1.5-3.0) for D-dimer (both p<0.001), adjusting for treatment arm.

Conclusion: In START, higher average levels of inflammation over several years were associated with subsequent higher risk of AIDS, SNA or death during 6 years on continuous ART. ART deferral resulted in higher levels of inflammation, which may have contributed to higher clinical event rates after pts were switched to continuous ART. Results should be interpreted with caution, but emphasize the need to diagnose HIV early to facilitate early ART initiation.

Optimizing Long-Term Exercise Benefits for Older Adults With HIV: The MOVING Study
Matilde Sánchez-Conde, Jorge Diaz-Alvarez, Rafael Garcia-Molina, Jesus Fernandez-Luna, Marta Martinez, Brian B. Vazquez-Brolicer, Sara Martin-Colmenarejo, Pablo Ryan, Fernando Dondal, Fatima Branas
1Hospital Ramón y Cajal, Madrid, Spain, 2Hospital Universitario Infanta Leonor, Madrid, Spain

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Background: We have previously demonstrated the efficiency of a 12-week multicomponent exercise program (MEP) in reversing frailty, improving physical performance, and preserving muscle mass in older adults with HIV (OAWH). The objective of this study was to analyze the efficacy of this MEP in the long term, after 1 year.

Methods: Sedentary adults 50 or over with and without HIV were included in a prospective longitudinal study with 12-, and 48-week follow-up. The intervention was a MEP performed in real-life conditions. Dependent variables were frailty (frailty phenotype), physical function (gait speed, SPPB), physical performance, and anxiety and depression (HADS and Geriatric Depression Scale Short-Form). Pre- and post-intervention measurements were analyzed using McNemar’s test for categorical variables and the Wilcoxon signed-rank test for quantitative variables.

Results: Sixty participants were included: 40 OAWH and 20 older adults without HIV. Median age was 56.5 years. 23.3% were women. The prevalence of frailty was 6.6% with no frail HIV-negative participants. At week 48, 32 participants continued, 21 OAWH and 11 older adults without HIV. The percentage of robustness increased after the MEP from 25.81% to 77.42% (RR 3 (95% CI, 1.7 – 5.2)), the percentage of prefrailty decreased from 64.52% to 22.58% and there were no frail patients at week 48. All participants improved (48-week vs baseline) their SPPB score (12 (11-12) vs 11 (10.5-12), p = 0.0001), and upper extremity strength [17 (15-20) vs 13 (12-15), p = 0.0001]. Anxiety (6.5 (4.5-11.5) vs 6 (3-10), p = 0.001) was improved only in the OAWH. In participants with an adherence ≥ 50%, the following were significantly improved (48-week vs baseline): gait speed (m/s) [1.29 (1.51-1.12) vs 1.33 (1.02), p = 0.02] without differences by HIV status, and aerobic endurance [74 (55-94) vs 61 (52-71), p = 0.001] only in the OAWH. Participants with an adherence ≥ 50%, showed a significant improvement 48-week vs 12-week was found in gait speed [1.29 (1.51-1.12) vs 1.21 (1.39-0.7), p = 0.03], upper limb strength [18 (16-21) vs 16.5 (15-19), p = 0.001], aerobic endurance [74 (55-94) vs 64 (59-83), p = 0.04], and agility [5.77 (5.2-6.7) vs 6.38 (5.7-6.8), p = 0.04].

Conclusion: A 48-week MEP improves robustness, physical function, physical performance in older adults independently of HIV status, and anxiety only in OAWH. Continuing the MEP after the first 32 weeks for up to one year produces an even greater improvement in physical function and physical performance.

839 Clonal Hematopoiesis and HIV Infection Are Associated With Geriatric Outcomes: The ARCHIVE Study

Win Min Han1, Mark Bloch1, David A. Baker1, Norman Roth2, Jennifer F. Hoy3, Ian Woolley4, Robert Finlayson5, Jane Costello6, Mark Dawson7, Sarah-Jane Dawson8, Mark Polizzotto9, Kathy Petoumenos10, Paul Veh11, Nile J. Dharan2, for the ARCHIVE Study Group

Background: Here we describe clinical and geriatric outcomes in the ARCHIVE cohort, a longitudinal cohort of older HIV-infected (HIV+) and HIV-uninfected (HIV-) adults with the objective to better understand HIV-related aging and potential interventions to improve quality of life (QoL) and reduce geriatric outcomes.

Methods: The ARCHIVE Study was a prospective cohort study established in 2012. Participants were recruited from 6 CFAR Network of Integrated Clinical Systems (CNICS) centers in the United States and Australia, and included older adults with HIV and without HIV. Assessments of QoL, using the EQ-5D Visual Analog Scale, and frailty, using a validated, modified frailty phenotype based on 4 Fried frailty components, were collected at routine clinical visits every ~3-6 months. Linear mixed models were used to assess individual level associations between frailty and QoL over time. Multivariable logistic and linear regression was used to evaluate for associations between HIV and CH, and geriatric outcomes.

Results: Of 344 participants, 173 had HIV and 169 did not. The median (interquartile range; IQR) age was 67 (62,72) years, 97% identified as male, 61% had a body mass index ≥25, 41% reported ever smoking, and 54% reported being diagnosed with cardiovascular disease. Overall, 23% had at least one CH mutation (27% of those with HIV and 18% of those without HIV; p = 0.045), 7% were frail, the median (IQR) PAA was 0.6 years (-2.5, 5.0), and the median (IQR) QoL score was 36 (29,39) out of 57. Participants with HIV had a median (IQR) duration of HIV of 27 (18,33) years and a median (IQR) CD4 nadir of 246 (143,372) cells/μL; all but one participant had a suppressed viral load. In adjusted regression analyses (Figure), HIV infection was independently associated with PAA (coefficients 1.73, 95% confidence interval [CI] 0.3-3.16, p = 0.02) and CH was independently associated with reduced QoL (coefficients -2.18, CI -3.32, -0.44, p = 0.01) and being frail (vs. pre-frail/robust; odds ratio 2.36, CI 1.01-5.63, p = 0.049).

Conclusion: In the ARCHIVE cohort, HIV was associated with PAA and CH, and CH was associated with frailty and reduced QoL. While our results warrant further exploration, they suggest that CH may be used as a biomarker for geriatric outcomes that may prioritize interventions for interventions designed to reduce geriatric outcomes and promote healthy aging.

840 Frailty and Health-Related Quality of Life in an Aging Cohort of People With HIV, 2012-2022

Lydia N. Drumright1, Bridget Whitney1, Crystal Chapman Lamberti1, Amanda Willig1, Robin M. Nance1, Stephanie A. Ruderman1, Sonia Napravnik1, Katerina Christopoulos1, Edward Cachay1, Lara Haidar2, Jimmy Ma3, Mari Kitahata4, Allison R. Weibel5, Heidi M. Crane6

1University of Washington, Seattle, WI, USA, 2University of Alabama at Birmingham, Birmingham, AL, USA, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 4University of California San Francisco, San Francisco, CA, USA, 5University of California San Diego, La Jolla, CA, USA, 6University of Manitoba, Winnipeg, Canada

Background: Due to advancements in antiretroviral therapy, people with HIV (PWH) are living near normal lifespans; however PWH age more rapidly and experience frailty at an earlier age than the general population. We examined the impact of frailty on quality of life (QoL) over time among PWH.

Methods: We assessed changes in QoL and frailty from 2012-2022 among PWH in care at 6 CFAR Network of Integrated Clinical Systems (CNICS) sites. Assessments of QoL, using the EQ-5D visual analog scale, and frailty, using a validated, modified frailty phenotype based on 4 Fried frailty components, were collected at routine clinical visits every ~3-6 months. Linear mixed models were used to assess individual level associations between frailty and QoL over time. Linear regression of mean QoL by frailty category and year was used to assess population level changes.

Results: 12,397 PWH with 38,830 frailty assessments were included. At initial frailty assessment, mean age was 45 years, 13% were female, 52% reported non-White race/ethnicity, 41% were robust (i.e., were not frail/prefrail), 45% prefrail, 14% frail, and mean QoL was 73. PWH who were prefrail and frail reported a 10% (95% CI: -10.4, -9.6, p < 0.001) and 24% (95% CI: -24.4, -23.0, p < 0.001) lower QoL respectively than those who were robust after controlling for age, sex, race/ethnicity, and COVID-era (i.e., March 2020 onward). Overall, we observed an annual increase in QoL at 0.4% until 2020, then a 2% decline during the COVID-era. Similar trends were observed for population level changes in QoL, with prefrail and frail PWH having a lower mean QoL, 13% (95% CI: -14.0, -11.5, p < 0.001) and 31% (95% CI: -32.0, -29.0; p < 0.001) respectively compared with robust PWH (Figure).

Conclusion: In our study, PWH who were frail and prefrail reported ~30% and ~10% lower QoL respectively than those who were not frail. While QoL among PWH in care in the United States appears to be slowly increasing over time, frailty can have a significant impact on QoL. Minimizing and preventing frailty and addressing comorbidities that contribute to frailty could increase QoL among PWH.
841 Dasatinib + Quercetin Drugs Reduced Senescent-Associated Secretory Phenotype (SASP) in PLWH PBMCs

Núria Climent, Victor Casanova, Andrea Rodriguez Agustín, María José Maleno, Cristina Rovira, Josep Mallools, Juan Ambrosioni, Sonssoles Sánchez-Palomino, José M. Miró, José Alcamí
Hospital Clinic of Barcelona, Barcelona, Spain

Background: Despite ART virological suppression, PLWH have increased inflammation and age-related diseases. Research in cellular aging has identified key biomarkers that define senescent cells (SC) which can be eliminated by senolytic drugs. These biomarkers include SA-βGal and mediators such as MCP-1, IL-6 or IL-6 that are principal components of the Senescent Associated Secretory Phenotype (SASP). We previously found that HIV-1 infection increased SC biomarkers such as SA-βGal, Bcl-2 and IL-6 in CD4+ T cells. The role of HIV-1 in promoting cellular senescence by SASP and soluble immune checkpoint mediators is not fully understood and could be key to develop new treatments for HIV-associated aging comorbidities. We aim to determine SASP mediators, SC linked soluble immune checkpoints (IC) and to know whether Dasatinib plus Quercetin (D+Q) senolytic drugs can influence SASP levels.

Methods: PLWH from acute, chronic and advanced infection cohorts before and after a year on ART and HIV-negative control group (NC) matched by sex and age were included (n=12). D+Q senolytic drugs were added to an ex vivo 3-day culture of PBMC with IL-2 from those cohorts. A set of 34-top SASP and 6 IC mediators were quantified by a customized Luminex in plasma and cell culture supernatants. Unpaired or paired non-parametric T-test and Pearson correlation were performed.

Results: SASP (IL-6, IL-8, IL-10, RANTES, GRO-α, TNF-RI/RII, CD30, CD30L, VEGF-A) and IC (PD-1, PD-1, PD-1, PD-2, LAG-3, CTLA-4) mediator levels were higher in advanced and chronic patients than in NC (p<0.05). These mediators were only reduced to NC levels by a year of ART in the acute cohort. IL-6 and SA-βGal in CD4+ T cells levels positively correlated with SASP mediators and IC such as IL-10, MIP-1α, PD-1, CTLA-4 and LAG-3 (p<0.05). D+Q ex vivo treatment decreased SASP mediators such as MCP-1, IL-8 (Figure), IL-6, IL-10, MIP-1α, IL1-RA, IL-1β, IL-1α, suPAR, PAI-1, tPA, MMP-1, MMP-12, HGF, Leptin, PIGF-1, MCP-4, GM-CSF, TNF-RI, Mip-3α, IL-7 or IL-15 (p<0.05) in non-ART treated PLWH. D+Q drugs in ART PLWH also decreased the above mentioned SASP mediators including MCP-1 and IL-8 (Figure)

Conclusion: Advanced and chronic PLWH with or without ART showed higher levels of SASP and immune checkpoint mediators. Ex vivo D+Q senolytic treatment decreased the majority of SASP factors analysed, normalizing these levels. Dasatinib could be useful to reverse cellular senescence, chronic inflammation and aging comorbidities from PLWH.

842 Neighborhood Vulnerability Predicts Non-Communicable Disease Risk Among People With HIV

Aiwei Yan1, Aihua Bian1, Peter Rebeiro2, Megan Turner2, Panidhi Ranadive3, Chandler Shaffermonk4,ustin Katona5, Noelle Best6, Victor Tian1, Leslie J. Pierce1, Timothy R. Sterling1, Bryan E. Shepherd1, Jessica L. Castilhoro1, Aima Ahonkhai1
1Vanderbilt University Medical Center, Nashville, TN, USA, 2Vanderbilt University, Nashville, TN, USA, 3Massachusetts General Hospital, Boston, MA, USA

Background: Social determinants of health underly persistent disparities in the rates of non-communicable diseases (NCDs) across communities but have been understudied in people with HIV (PWH).

Methods: We assessed associations between the CDC’s Social Vulnerability Index (SVI) and NCD burden and risk in PWH by using data at the Vanderbilt HIV Clinic from Jan 2009-Dec 2019. The SVI is a census-tract-level measure of deprivation based on 16 factors, grouped into a total score and four themes (socioeconomic status, household composition/disability, minority status/language, and housing/transportation); higher SVI indicates greater neighborhood vulnerability. SVI was assigned using residence at enrollment or first visit after Jan 2009. NCDs included cardiovascular, liver, metabolic, and chronic kidney disease (stage ≥3), as well as non-AIDS defining cancers. Multivariable proportional odds logistic and Poisson regression models were used to assess the relationship between baseline SVI (total and themes) and both baseline and longitudinal NCDs (respectively), accounting for clustering and adjusting for age, substance use, gender/HIV risk factor, race/ethnicity, prevalent NCDs, antiretroviral therapy, depression, HIV RNA, hepatitis C, year, CD4 count, time since HIV diagnosis, and body mass index. Continuous variables including SVI were modeled using restricted cubic splines with 3 knots.

Results: Among 4440 PWH, median age was 41 years, 59% were men who have sex with men, 21% were cis-gender women, 41% were non-Hispanic Black, and 50% were non-Hispanic white. Median total SVI was 0.6 (IQR: 0.3–0.8) and median follow-up was 2.6 years (IQR: 1.1–5.4). At baseline, 44% had ≥1 NCD and 19% had ≥2 prevalent NCDs. 24% PWH without prevalent NCDs, 32% developed NCDs during follow-up. 1916 patients with prevalent NCDs, 41% developed additional NCDs. Lower SVI was associated with lower number of prevalent NCDs (Figure 1a). In separate analyses, SVI socioeconomic status and household composition/disability themes were also associated with prevalent NCD burden (all p<0.05). In longitudinal analyses, there was no significant association between SVI and the risk of incident NCDs (Figure 1b).

Conclusion: Neighborhood vulnerability was associated with NCD burden among PWH, even after adjusting for individual characteristics, though confidence intervals included the null for incident NCDs. Relationships between neighborhood-level social determinants of health and NCD comorbidity among PWH warrant further study.

843 Application of STOPP Criteria in an Urban Cohort of People Aging With HIV

Lauren F. O’Connor1, Jenna Resnik1, Sam Simmons, Vinay Bhandaru1, La Marcus Wingate1, Debra Benator2, Amanda Castel1, Anne Monroe1, for the DC Cohort Executive Committee
1George Washington University, Washington, DC, USA, 2Howard University, Washington, DC, USA

Background: The validated Screening Tool of Older People’s Prescriptions (STOPP), which identifies potentially inappropriate prescribing (PIP), i.e., treatment for which the potential risk outweighs the potential benefit, may be particularly important for aging people with HIV (PWH) and comorbidities. PIP may exacerbate symptoms and worsen adherence, which is particularly relevant among PWH. Our objectives were to: (1) Apply STOPP to identify PIP among an urban cohort of PWH aged >50 years with >1 comorbidity; (2) Describe correlates of PIP; (3) Evaluate the relationship between PIP, symptom burden and quality of life (QOL).

Figure 1. D+Q senolytic drugs reduced the expression of SASP factors by Luminex quantification.
Methods: We analyzed data from the DC Cohort, a multicenter longitudinal study of 12,000 PWH receiving care in Washington, DC. Participants who completed a survey on symptom burden, social determinants of health (SDOH), and QOL were included. Symptom burden was measured with a continuous global distress index. We used STOPP to identify PIP and evaluated unadjusted/adjusted logistic regression models to determine demographics, comorbidities, HIV clinical factors, and SDOH associated with PIP. We then used structural equation modeling to evaluate whether symptom burden mediates the relationship between PIP and QOL.

Results: Of the eligible DC Cohort participants (n=3, 902 (%)) completed surveys and 511 (56.6%) had STOPP-designated PIP. The most common PIP were related to the musculoskeletal (n=349 (38.7%)), central nervous (n=135 (37.1%)), and urogenital (n=259 (28.7%)) systems. The strongest predictor of PIP was hypertension (aOR (95% CI): 5.17 (3.15, 8.51)). Non-Hispanic White participants were significantly more likely to have PIP than Non-Hispanic Black participants (aOR: 2.45 (95% CI: 1.42, 4.23)). Older age, having a housing or utility need, or receiving HIV care at a hospital versus a community site were all significantly associated with increased odds of PIP. In mediation models, symptom burden was a statistically significant mediator, but the direct effect (non-mediated) of PIP on QOL was not significant.

Conclusion: We found that over half of our participants had a PIP and hypertension is a strong predictor of PIP. Future interventions should use STOPP as a means of alerting clinical teams to clinically relevant PIP, especially among the high-risk groups identified here.

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844 Effects of HIV Status on Incidence of Post-COVID Conditions in Patients With SARS-CoV-2 (COVID-19)

Michael A. Horberg1, Celeena R. Jefferson1, Eric S. Watson1, Lily F. Fathi1, Seohyun S. Kim1, Kelly A. Gebo2, Brenna Hogan1, Elizabeth Humes3, Keni R. Althoff4
1Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 3The Johns Hopkins University School of Public Health, Baltimore, MD, USA, 4The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: SARS-CoV-2 (COVID-19) has been linked to long-term effects known as Post-COVID Conditions (PCC), which present post-acute COVID infection. While many studies have estimated PCC incidence, few have focused on immunocompromised patients, such as people with HIV (PWH). We estimated PCC incidence among PWH compared with people without HIV (PWoH) within Kaiser Permanente Mid-Atlantic States (KPMAS), an integrated closed healthcare system with high ascertainment of COVID-19 testing, vaccinations, and diagnoses among our patient membership.

Methods: Using KPMAS electronic health records, we identified adult patients (≥18 years) with a positive polymerase chain reaction (PCR) test for COVID between 1/1/2020-1/31/2022. PWH, identified from the KPMAS HIV registry, were matched to PWoH by month of first PCR-positive test, age group (deciles), race, sex, vaccination status prior to test, and service area (to account for physician diagnostic variation), using 1:3 variable ratio matching. Patients were considered to have PCC if diagnosed with one of 17 previously-identified PCC-related conditions incident in the 30-180 days post first positive test date. From our earlier work, PCC-related conditions included anosmia, cardiac dysrythmia, diabetes, gastrointestinal, neurologic, and genitourinary disorders, malaise, and non-specific chest pain. Relative risk with 95% confidence intervals were calculated and the association between HIV and PCC was evaluated with Rao-Scott Chi-Square (significance at p<0.05), weighted to account for disproportionate matching.

Results: We matched 749 PWH to 2,236 PWoH with >98% having ≥1 matching (1.3 = 740; 1.2 = 7, 1 = 2; [table]). The matched cohort was predominately black (80%) and male (64%) with a median age of 47 years. Overall, 22% of PWH and 19% of PWoH developed PCC. Risk of incident PCC among PWH (vs PWoH) was 19% higher (RR = 1.19; 95% CI: 1.01-1.40 p = 0.035). While PCC incidence was higher among PWH with CD4 ≤200/µL (37%, among 27) compared to those with CD4 >200/µL (22% among 633, p = 0.07), PCC incidence did not vary by viral suppression status (> or ≤200 copies/ml; 22%, p = 0.95).

Conclusion: We found PWH are at greater risk for PCC compared to PWoH, contributing evidence for enhanced care of immunocompromised populations who are infected with COVID. Assessment for PCC 30-180 days post-COVID, especially among PWH, should be a best practice. Further research into specific PCC conditions, effects of vaccination, and COVID disease severity among PWH is needed.

845 Evaluating the Effect of Vaccination on Post-COVID Conditions Among Patients With and Without HIV

Michael A. Horberg1, Eric S. Watson1, Celeena R. Jefferson2, Lily F. Fathi1, Seohyun S. Kim1, Kelly A. Gebo2, Brenna Hogan1, Elizabeth Humes3, Keni R. Althoff4
1Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA, 2The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 4The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: Previous studies have described conditions presenting post-acute SARS-CoV-2 (COVID) infection, known as Post-COVID Conditions (PCC) or as Long COVID; but the effect of vaccination, especially among immunocompromised patients, is unclear. We evaluated the effect of COVID-19 vaccination on PCC within an integrated health system (Kaiser Permanente Mid-Atlantic States; KPMAS), for patients with HIV (PWH) and without HIV (PWoH). KPMAS is a closed healthcare system with high ascertainment of COVID-19 diagnostic and vaccination records.

Methods: Using KPMAS electronic health records, we identified adult patients (≥18 years) with a positive SARS-CoV-2 PCR test result between 1/1/2021-1/31/2022. The first positive result in this period defined the index date. Vaccination was defined as having ≥2 doses of mRNA or 1 for J&J COVID-19 vaccine ≥14 days prior to the index date. AHRO Clinical Classification Software was used to group ICD-10 codes for 17 previously-identified PCC-related conditions, including anosmia, cardiac dysrythmia, diabetes, gastrointestinal, neurologic and genitourinary disorders, malaise, and non-specific chest pain. PCC was defined as having an incident diagnosis of any of these conditions, 30-180 days after index. Poisson regression models with robust variance regression models determined if the relative risk of PCC by COVID-19 vaccination status was the same among PWH (from KPMAS HIV registry) and PWoH (without HIV (PWoH)). PCC is a closed healthcare system with high ascertainment of COVID-19 diagnostic and vaccination records.

Results: Over 72,376 patients were identified, including 429 PWH and 71,884 PWoH, of whom 39% and 44% were unvaccinated, respectively (table). No interaction was found between HIV and vaccination (p value = 0.81). After removing the interaction term, PWH showed significantly increased risk versus PWoH (RR 1.25; p = 0.01), while unvaccinated patients versus vaccinated did not (RR 1.02; p = 0.27). Sex, age, service area, and race all showed significant risk ratios. Men showed lower risk (RR = 0.78) compared to women, while greater age (RR = 1.52 85+; Ref = 18-29), non-white race and Hispanic ethnicity (RR = 1.14 Hispanic; Ref = NH White) showed increased risk. Conclusion: After adjusting for demographic characteristics, risk of PCC did not differ by vaccination status among PWH or PWoH. These findings may suggest COVID-19 vaccination is not protective against PCC after breakthrough infection. The figure, table, or graphic for this abstract has been removed.

846 Post-Acute Sequelae of COVID-19 in an Early-Treated HIV Cohort in Thailand

Ferron F. Dcampo1, Tyler Hamby2, Carlos F. Saldanha3, Parini Sithinamsuwan4, Suteerapon Pinyakorn4, Varaporn Unosomb4, Somchai Siriplenchit, Nittaya Phanuphak4, Robert Paut1, Phillip Chan4, Sandhya Vasan4, Lydie Trautmann4, Serena Spudich4
1University of California, Los Angeles, Los Angeles, CA, USA, 2Yale University, New Haven, CT, USA, 3Pharmangkukkhol College of Medicine, Bangkok, Thailand, 4Institute of HIV Research and Innovation (IHRI), Bangkok, Thailand, 5University of Missouri St Louis, St Louis, MO, USA, 6Yale University, New Haven, CT, USA

Background: People with HIV (PWH) may be at elevated risk of post-acute sequelae of COVID-19 (PASC), but PASC frequency and risk factors in PWH who initiated antiretroviral therapy (ART) during acute HIV (AHI) is unknown. We assessed pre-AHI characteristics and compared immunologic and neuropsychiatric outcomes of participants with and without PASC in the RV254 AHI study in Thailand.
Methods: Participants enrolled and initiated ART during AHI, with standardized longitudinal assessment of blood T cell counts and viral load (VL), cognition (Color Flairs 1 & 2, Grooved Pegboard, Trail Making A), and mood (Hospital Anxiety & Depression Scale, Patient Health Questionnaire-9). By 7/2023, those ≥1 year after confirmed COVID were stratified as no PASC or PASC by persistence or occurrence of ≥1 PASC symptom (on a PASC symptom questionnaire) ≥3 months after acute COVID, lasting for >2 months. Demographic characteristics, COVID-19 course (time period/variant, number of infections, and hospitalization), and immunologic, cognitive, and mood parameters pre- and post-COVID-19 were compared in PASC vs no PASC using non-parametric methods.

Results: 216 RV254 participants were assessed a median 15[1QR 13-17] months after acute COVID; 55(25%) had experienced PASC and 15(7%) had ongoing symptoms. Common symptoms were fatigue (55%), exercise intolerance (25%), sleep disturbance (15%), cough (13%), and memory impairment (13%). PASC vs no PASC had similar pre-COVID parameters including median age 30 vs 31 years; 95% vs 98% male; CD4+ count 706 vs 701 cells/µl; pre-COVID VL >50 cpsi/ml 0% vs 1%; duration from AHI to acute COVID 6.0 vs 5.4 years (all p>0.05). Those with PASC had a higher pre-COVID frequency of moderate-to-severe anxiety (HADS-A>11), fewer COVID vaccinations, and higher hospitalization rates with longer median hospital stay (Table 1). No differences were detected in variant type, number of infections, or immunologic and neuropsychiatric measures pre- to post-COVID between PASC and no PASC participants.

Conclusion: In this cohort of young mostly male PWH on suppressive ART initiated during AHI, ≥25% experienced PASC and 7% had ongoing symptoms >1 year after documented COVID-19. Immunologic and neuropsychiatric changes pre- and post-COVID did not differ in participants with and without PASC. Higher pre-COVID anxiety severity, frequency of COVID hospitalization, and fewer COVID vaccinations associated with PASC, suggesting opportunities to prevent PASC in PWH including mental health interventions and vaccination

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<th>Table 1: Characteristics of RV254 Participants with and without post-acute sequelae of COVID-19 (PASC) based on non-parametric survival analysis</th>
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<td>Age (years)</td>
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847 Post-COVID Conditions Following COVID-19: A Matched Analysis of Patients With SARS-CoV-2
Deborah E. Malden1, In-Lu Amy Liu1, Lei Qian1, Sara Y. Tartof1, Xiaoming Li2, Deborah E. Malden1, In-Lu Amy Liu1, Lei Qian1, Sara Y. Tartof1, Xiaoming Li2, Xiaoming Li2
1Kaiser Permanente Southern California, Pasadena, CA, USA; 2Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: COVID-19 vaccinations protect against severe illness and death, but associations with post-COVID conditions (PCC) are less clear. We aimed to evaluate the association between prior vaccination and new-onset PCC among individuals with SARS-CoV-2 infection across eight large healthcare systems in the United States.

Methods: This study was a retrospective matched cohort study using electronic health records (EHR) from patients of all ages with SARS-CoV-2 positive tests (PCR or antigen) during March 2021-February 2022. Vaccinated and unvaccinated COVID-19 cases were matched on location, test date, severity of acute infection, age, and sex. Vaccination status was ascertained using EHR and integrated data on externally administered vaccines. Adjusted relative risks (RRs) were obtained from Poisson regression. PCC was defined as a new diagnosis in one of 13 PCC categories from 30 days to 6 months following a SARS-CoV-2 positive test.

Results: The primary analysis included 161,531 COVID-19 cases among vaccinated patients and 161,531 matched unvaccinated cases. Compared to unvaccinated cases, vaccinated cases had a similar or lower risk of all PCC categories except mental health disorders (RR: 1.06; 95% CI: 1.02-1.10). Vaccination was associated with <10% lower risk of severe COVID (RR 0.90; 95% CI 0.86-0.95), circulatory (RR 0.88; 95% CI 0.83-0.94), blood and hematologic (RR 0.79; 95% CI 0.71-0.89), skin and subcutaneous (RR 0.69; 95% CI 0.66-0.72), and non-specific COVID-19 related disorders (RR 0.53; 95% CI 0.51-0.56). In general, associations were slightly stronger at younger ages but persisted regardless of SARS-CoV-2 variant period, number of vaccine doses received, or time since vaccination.

Conclusion: Pre-infection vaccination was associated with reduced risk of several PCC outcomes and hence may decrease the long-term consequences of COVID-19.

848 Long COVID Between People With and Without HIV: A Statewide Cohort Analysis
Xueying Yang1, Ziang Liu1, Jiajia Zhang1, Bankole Olatosi1, Sharon Weissman2, Xiaoming Li3
1University of South Carolina at Columbia, Columbia, SC, USA; 2University of South Carolina at Columbia, Columbia, SC, USA

Background: Evidence on stratification of long COVID symptoms by immunocompromise status is lacking. People with HIV (PWH) have been documented to have an elevated risk of severe COVID-19 outcomes, yet the data on long COVID among PWH are limited. Using a large, statewide sample dataset, this study aims to characterize and compare the risks of a panel of post-acute sequelae of COVID-19 between PWH and non-PWH comparison group.

Methods: Using an integrated statewide electronic health record data from HIV cohort and COVID-19 tester cohort, we were able to identify COVID-19 positive individuals by HIV status between March 02, 2020 and April 14, 2022 in South Carolina. Using the COVID-19 diagnosis date as the index date, a total of 132 individual long COVID sequelae were ascertained through ICD-10 codes until the end of the cohort. Risks of individual sequelae were compared between PWH and non-PWH groups. For each long COVID condition, we examined diagnoses as outcomes separately and only excluded the specific diagnosis one year before the index date for models examining that same outcome in both PWH and non-PWH. We employed logistic regression models to estimate the odds ratio of the risks of individual sequelae between case and control groups, adjusting for socio-demographics and comorbidities.

Results: Among a total of 1,351,489 COVID-19 positive individuals, 3,485 were PWH and 1,348,004 were PWoH. PWH were generally older than PWoH (47 vs 36 years old), had a higher proportion of male (64% vs 45%) and Black population (73% vs 26%). The prevalence of any long COVID condition was 58.68% and 33.80% for PWH and non-PWH, respectively. After adjusting covariates, PWH were associated with a higher risk of 118 individual long COVID sequelae in nearly every organ system, such as Circulatory Disease (Encephalitis: adjusted odds ratio [aOR]: 5.86, 95%CI: 2.37-14.48), Mental, Behavioral and Neurodevelopmental Disorders (Stimulant-related disorders: aOR: 6.41, 95%CI: 3.31-6.43), Sedative-related disorders: aOR: 8.30, 95%CI: 1.31-7.78; Miscellaneous Mental and Behavioral Disorders/conditions: aOR: 3.03, 95%CI: 1.56-5.90), and Diseases of the Genitourinary System (Nephritis: aOR: 3.98, 95%CI: 1.75-9.06).

Conclusion: In this large observational study, PWH appears to have a higher risk of a variety of long COVID outcomes. These findings warrant further investigation in understanding how PWH leads to worse long COVID outcomes with more observational studies in persons with or without HIV.

849 COVID-19 Vaccine Protection Against Long COVID: A Population-Based Cohort Study
Xueying Yang1, Ziang Liu1, Jiajia Zhang1, Bankole Olatosi1, Sharon Weissman2, Xiaoming Li3
1University of South Carolina at Columbia, Columbia, SC, USA

Background: Prevention, Atlanta, GA, USA

Methods: This study was a retrospective matched cohort study using an integrated electronic health record system of SARS-CoV-2 positive test. Pre-infection vaccination was associated with reduced risk of several PCC outcomes and hence may decrease the long-term consequences of COVID-19.
850 Sexual Minority Adults Experienced More Severe and Longer COVID-19 Symptoms Than Heterosexual Adults

Xinyi Li1, Jincong Q. Freeman1, Yijin Xiang1, Yong G. Lee1, Victoria Umutoni1
1George Washington University, Washington, DC, USA, 2University of Chicago, Chicago, IL, USA, 3University of Southern California, Los Angeles, CA, USA, 4Watterson University, Newark, NJ, USA

Background: Lacking access to COVID-19-related care could prolong symptoms and accentuate adverse health outcomes among vulnerable populations, including sexual minority populations. We investigated the relationships between sexual orientation and COVID-19 diagnosis, symptoms, and vaccination status.

Methods: Data for this analysis were from the 2022 National Health Interview Survey. Sexual orientation was self-reported, categorized as sexual minority (self-identified as gay, lesbian, bisexual, or other) and heterosexual. Weighted percentages of self-reported COVID-19 diagnosis, symptoms, and vaccination status were tabulated by sexual orientation, with p-values computed using Rao-Scott Chi-squared tests. Separate weighted logistic regression models were fit to compare these outcomes by sexual orientation and to calculate adjusted odds ratios (aOR) and 95% confidence intervals (95% CI), controlling for age, race/ethnicity, education level, health insurance coverage, and general health status. All analyses accounted for complex sample design.

Results: Among a total of 246,508 subjects, the prevalence of any long COVID conditions was 26.03%, with no major differences across the pandemic waves (e.g., Omicron). With a reference group of unvaccinated individuals, individuals with any dose of vaccination were associated with lower odds of 68 individual sequelae conditions, which involves 11 organ systems. Such protective effect was even higher among individuals who received booster dose. For example, a decreased risk of the disease of the Respiratory System was observed for individuals with fully (adjusted hazard ratio [aHR]: 0.75, 95% CI: 0.68, 0.84) and booster vaccination (aHR: 0.60, 95% CI: 0.53-0.68). Likewise, a reduced risk was also observed for the diseases of the Circulatory System (fully: aHR: 0.88, 95% CI: 0.83-0.93; booster: aHR:0.72, 95% CI: 0.64-0.81), Skin and Subcutaneous Tissue (fully: aHR:0.75, 95% CI: 0.68, 0.84; booster: aHR: 0.52, 95% CI: 0.40-0.69).

Conclusion: In this statewide longitudinal observational study, we observed that fully or boosted vaccination appears to have some extent of protection against the development of multiple long COVID outcomes.

851 Persistence of Anxiety and Depression in US Adults by COVID-19 Vaccination Status, 2020-2023

Yanhan Shen1, Kate Pernose2, McKaylee Robertson1, Rachael Piltch-Loeb3, Sasha Fleary3, Sarah Kulkarni1, Chloe Teasdale4, William You1, Subha Balasubramanian5, Surabhi Yadav1, Bai Xi Jasmine Chan1, Milton L. Wainberg6, Scott Ratzani7, Denis Nash8, Angela Parcesepe9
1City University of New York, New York, NY, USA, 2Columbia University, New York, NY, USA, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: The COVID-19 pandemic has impacted mental health among adults in the US. However, little is known about the persistence of symptoms of anxiety and depression, over the three years since the emergence of SARS-CoV-2 or the relationship between such symptoms and COVID-19 vaccination. We described the persistence of anxiety and depression among a large cohort of US adults.

Methods: Participants from the CHASING COVID Cohort completed the Generalized Anxiety Disorder (GAD-7) and Patient Health Questionnaire (PHQ-8) at least one of 14 assessments, approximately every 3 months between July 2020 and June 2023. Persistent symptoms of anxiety or depression was defined as reported moderate/severe symptoms of anxiety (GAD-7>7) or depression (PHQ-8>8) for >=7 assessments. Participants were classified as vaccinated (or unvaccinated) if they reported any (or never) COVID-19 vaccine between December 2020 and June 2023. We conducted multiple imputations for 18.3% missing GAD-7 or PHQ-8 scores. We used latent class growth analysis (LCGA) to identify groups of participants with shared GAD-7 or PHQ-8 trajectories over 36 months of follow-up. Chi-square tests were used to compare sociodemographic factors among those with and without persistent symptoms of anxiety/depression and by LCGA groups. At each time point, log-binomial models were used to estimate the age- and gender-adjusted prevalence of moderate/severe anxiety or depression stratified by vaccination status.

Results: Among 5652 participants, prevalence of persistent symptoms of anxiety or depression was 19.0% (N=1074) and 22.8% (N=1288) over 36 months, respectively. Using LCGA model, 26.7% (N=1509) and 26.8% (N=1516) of participants had persistently high trajectory of GAD-7 or PHQ-8 over 36 months of follow-up. Chi-square tests were used to compare sociodemographic factors among those with and without persistent symptoms of anxiety/depression and by LCGA groups. At each time point, log-binomial models were used to estimate the age- and gender-adjusted prevalence of moderate/severe anxiety or depression stratified by vaccination status.

Conclusion: Further investigations are warranted to elucidate bidirectional relationships between COVID-19 vaccination and mental health symptoms, extending the extent to which moderate/severe mental health symptoms may be a barrier to staying up to date on COVID-19 vaccination.
852 Predictors of Long COVID in Adults With Untreated Mild-To-Moderate COVID-19

Teresa H. Evering, Pooja T. Sahu, Carlee Moser, Nikolaus Jilg, Rachel A. Bender Ignacio, Prasanna Jeganathan, Katyia Corrado, Kevin Wongsoirdjo, Justin Ritz, Jonathan Z. Li, Davey M. Smith, Michael D. Hughes, Judith S. Carrier, Kara W. Chew, for the ACTIV-2/A5401 Study Team

Woll Cornell Medicine, New York, NY, USA; [Harvard Chan School of Public Health, Boston, MA, USA; Massachusetts General Hospital, Boston, MA, USA; University of Washington, Seattle, WA, USA; Stanford University, Stanford, CA, USA; Hunter–UCLA Medical Center, Torrance, CA, USA; Brigham and Women’s Hospital, Boston, MA, USA; University of California San Diego, La Jolla, CA, USA; University of California Los Angeles, Los Angeles, CA, USA

Background: Identifying Long COVID predictors is crucial. We studied associations with Long COVID in untreated adults in the ACTIV-2/A5401 trial.

Methods: Non-hospitalized adults with COVID-19 randomized to placebo within 10 days of symptom onset were analyzed. Long COVID was defined as self-reporting overall COVID-19 symptoms as present in the 4 weeks preceding symptom onset. We also assessed 27 individual symptoms. Demographics, clinical and biomarker variables at day 0 (body mass index, smoking, COVID-19 vaccination, comorbidities, symptom score summed across 13 acute symptoms each graded as absent[0]/mild[1]/moderate[2]/severe[3], anterior nasal viral RNA levels, serostatus by anti-Spike and anti-nucleocapsid antibody), and inflammatory/coagulation markers (classified as above normal or tertiles of normal values) at days 0 and 28 were evaluated as predictors of Long COVID using regression models.

Results: 546/702 (78%) of participants who received placebo January-August 2021 completed LT diary at W24: median age 44 years, 53% female, 99% cis-gender, 13% Black, 53% Hispanic/Latino, 15% previously vaccinated, 49% with long COVID had significant lower levels of neutralizing antibodies against the D614G variant compared to the control cohort (p-value: 0.0064). However, children and adolescents with long COVID had significant lower levels of neutralizing antibodies against S protein.

Conclusion: Our study is the first to report antibody levels in CYP with long COVID. These results indicate that, in our cohort, CYP with long COVID had lower antibody response against the RBD of SARS-CoV-2, along with less neutralizing activity. Further experiments are needed to assess the reason and how this could impact the development of long COVID and its associated symptoms.

Importantly, the lower anti-RBD antibody response could provide a potential biomarker for the diagnosis of long COVID in the pediatric population.

853 Long COVID in Children Is Associated With Lower Anti-RBD IgG/IgA and Neutralizing Antibody Levels

Jon Izquierdo-Pujol, Sara Morón-López, Núria Pedreño, Tetyana Pidkova, Victor Urra, Judith Dalmau, Alba Gonzalez-Aumatell, Clara Carreras-Abad, Maria Mendez, Carlos Rodrigo, Julia Blanco, Jorge Carrillo, Benjamin Trinidi, Javier Martinez-Picardo, IrsCasita Institute for ABS Research, Badajoz, Spain, Hospital Germans Trias i Pujol, Barcelona, Spain

Background: Most people recover quickly after SARS-CoV-2 acute infection; however, a significant percentage can develop long-term persistent symptoms, a condition known as long COVID, post-acute sequelae of SARS-CoV-2 (PASC) or Post-COVID-19 Condition (PCC). One of the main hypotheses on the underlying mechanism is the dysregulation of immune and inflammatory responses that persists after the acute infection. Most studies are focused on adults, neglecting cases in children and young people (CYP), where mechanisms may differ. Here, we analyzed the humoral and neutralizing antibody response in pediatric population with and without long COVID.

Methods: We analyzed 131 blood samples from the pediCOVID cohort (Hospital Germans Trias i Pujol), which includes 108 children/adolescents diagnosed with long COVID and 23 control CYP. An in-house ELISA was used to measure SARS-CoV-2 specific IgG and IgA antibody plasma levels (Anti-S2, Anti-RBD and Anti-N). In addition, neutralizing antibody levels were measured using a luciferase-reporter lentiviral pseudovirus assay, expressing SARS-CoV-2 S protein.

Results: The long COVID cohort had a median age of 14.3 (IQR, 12.5-15.2) and 69.1% of female participants (no differences with control cohort). Patients had a median of 10 symptoms associated with long COVID (IQR, 7-16). The most common symptoms were asthenia/fatigue, (98%), neurocognitive disorders (84%) and brain fog (82%). CYP with long COVID had significant lower levels of both anti-RBD IgG and IgA antibodies compared to the control cohort (p-value < 0.0001 and 0.0332, respectively). Consistently, children and adolescents with long COVID had significant lower levels of neutralizing antibodies against the D614G variant compared to the control cohort (p-value: 0.0064). However, plasma levels of both anti-S2 and anti-N antibodies remained similar to the control group.

Conclusion: Our study is the first to report antibody levels in CYP with long COVID. These results indicate that, in our cohort, CYP with long COVID had lower antibody response against the RBD of SARS-CoV-2, along with less neutralizing activity. Further experiments are needed to assess the reason and how this could impact the development of long COVID and its associated symptoms.

Importantly, the lower anti-RBD antibody response could provide a potential biomarker for the diagnosis of long COVID in the pediatric population.
855 Relationship Between Serum Cortisol Level and Long COVID Symptoms in Post-Acute COVID-19
Thomas Dalhuisen, Joshua Hauser, Scott Lu, Lucas Kallas Silva, Rebecca Hoh, Steven G. Deeks, J. D. Kelly, Jeffrey Martin, Peter W. Hunt, Elizabeth Murphy, Timothy J. Henrich, Morrie Schambelan, Michael J. Peluso, for the LIINC Study Team

University of California San Francisco, San Francisco, CA, USA

Background: Low cortisol levels have been reported in some people with Long COVID (LC), but this observation has yet to be confirmed. Some people with LC meet the definition of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), an illness that has also been associated with cortisol dysregulation. Further evaluation of cortisol post-COVID could better define LC biology.

Methods: We evaluated 364 individuals in the UCSF LIINC post-COVID study cross-sectionally, 3-6 months after SARS-CoV-2 infection. We defined LC as ≥1 COVID-associated symptom (n=144), and non-LC (nLC) as no symptoms (n=56). We further defined those with LC who met Institute of Medicine ME/CFS criteria at 3 months as LC-ME (n=28) and those with LC but without LC-ME as LC-nME (n=116). We measured cortisol in serum collected 8AM-12PM on day of assessment and performed analyses stratified by collection time.

Results: The cohort was 55% female, median age was 43. Cortisol levels (µg/dL) did not differ between LC and nLC groups (median 9.9 vs 8.8) when aggregated across collection time. Proportions of subnormal cortisol levels (<5) were similar (9.7% vs 8.9%). When stratified by collection time, 8-9AM cortisol levels tended to be lower in those with LC compared to nLC (12.4 vs 14.9, p=0.36), and 9-10AM cortisol levels tended to be higher comparing LC to nLC (10.3 vs 8.5, p=0.15), albeit neither difference was statistically significant. No differences were observed between 10-11AM and 11AM-12PM. When stratified by post-COVID ME status, cortisol levels between 8-9AM were significantly lower in the LC-ME group compared to the nLC group (8.2 vs 14.8, p=0.02), even after adjusting for age, sex and BMI (Fig 1). Conversely, cortisol levels between 9-10AM were significantly higher in the LC-ME group compared to the nLC group (13.7 vs 8.5, p=0.02), also after adjusting for age, sex, and BMI (Fig 1). No differences were observed comparing LC-nME to nLC.

Conclusion: We found that cortisol levels tended to be lower between 8-9AM and to be higher between 9-10AM in those with LC, and that this difference appears to be driven by those with LC-ME, consistent with prior observations of a delayed cortisol peak in ME/CFS. These findings significantly add to our understanding of cortisol in LC and highlight the importance of considering collection time. Longitudinal measures of cortisol in individuals with LC will be critical to further inform the biology of the condition.

Figure 1: (A) Heatmap demonstrating individual demographics and hierarchical clustering of biomarkers for each individual. (B) Comparisons of clinical demographics between clusters.

856 Association of Long COVID With Health-Related Quality-of-Life Outcomes
Malini M. Gandhi, Carlee Moser, Judith S. Currier, Justin Blitz, Joseph J. Ervin, Eric Daar, David Wohl, William Fischer, Upinder Singh, Michael D. Hughes, Davey M. Smith, Teresa H. Evering, Kara W. Chew, for the ACTIV-2/A5401 Study Team

Harvard Medical School, Boston, MA, USA, Harvard TH Chan School of Public Health, Boston, MA, USA, University of California Los Angeles, Los Angeles, CA, USA, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, Harbor-UCLA Medical Center, Torrance, CA, USA, Stanford University, Stanford, CA, USA, University of California San Diego, La Jolla, CA, USA, Weill Cornell Medicine, New York, NY, USA

Background: Long COVID is a significant and growing public health burden. The association of Long COVID with health-related quality-of-life (HRQOL) has not been well-characterized.

Methods: Participants (N=546) who received blinded placebo in the ACTIV-2/ A5401 outpatient COVID-19 treatment trial with symptom diary data at week 24 were assessed for Long COVID, defined as presence of self-assessed COVID-19 symptoms within the last 4 weeks at the week 24 visit. HRQOL at week 24 was evaluated by the EQ-SD-SL (EQ-SD) and SF-36v2 (SF-36) questionnaires. Modified Poisson regression and Wilcoxon rank-sum tests compared EQ-SD and SF-36 measures between participants with vs without long COVID.

Results: This cohort enrolled between January and August, 2021; median age was 44 years, median time from symptom onset was 5 days; 53% female, 99% cisgender, 80% White, 13% Black, 53% Hispanic, 58% high-risk for severe COVID-19, 36% with Delta and 64% pre-Delta variant infection, 16% vaccinated, and 52% anti-nucleocapsid or anti-spike antibody positive. Long COVID was present in 13.6% (74/546) of participants. EQ-SD was completed by 80% and SF-36 by 81%. A higher frequency of participants with vs without long COVID was assessed by the EQ-5D-5L (EQ-5D) and SF-36v2 (SF-36) questionnaires.

Conclusion: Long COVID is a significant and growing public health burden. The association of Long COVID with health-related quality-of-life (HRQOL) has not been well-characterized.
reported problems in each of the five EQ-5D dimensions; mobility (24% vs 6%, \(p<0.001\)), self-care (8% vs 2%, \(p=0.007\)), usual activities (38% vs 6%, \(p<0.001\)), pain/discomfort (57% vs 14%, \(p<0.001\)), and anxiety/depression (41% vs 15%, \(p<0.001\)), with risk ratios of 2.75 to 6.18 (Figure 1A). Participants with vs without long COVID had lower scores on EQ-5D visual analogue scale, in which current health was self-rated from 0 (worst) to 100 (best) (median 85 vs 95, \(p<0.001\)). Participants with long COVID also had lower scores in each of the eight SF-36 domains (general health, physical functioning, physical role, bodily pain, vitality, social functioning, emotional role, and mental health; all \(p<0.001\)) (Figure 1B) and composite physical and mental component scores (both \(p<0.001\)).

**Conclusion:** Long COVID is associated with worse HRQOL outcomes across multiple domains, highlighting the need to develop preventative and therapeutic interventions for this condition.

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857 **Predictors of Breakthrough COVID-19 Infection From the STOPCoV Cohort**

Sharon Walmsley, Majid Nabipoor, Leif Erik Lovblom, Rizani Ravindran, Roaya M. Dayam, Dorin Manase, Karen Colwill, Anne-Claude Gingras, for the StopCov Research Team

**University of Toronto, Toronto, Canada**

**Background:** Our current understanding of the human immune response to SARS-CoV-2 does not enable us to quantify the degree of protection COVID-19 vaccines confer against infection and transmission especially with emerging variants.

**Methods:** The STOPCoV (Safety and effectiveness of Preventative COVID Vaccines) study is an ongoing prospective decentralized study with the primary aim of comparison of the antibody responses to COVID vaccines in those aged > 70 years relative to a younger cohort aged 30-50 years. In this analysis, we assessed predictors of breakthrough COVID-19 infection during the Omicron BAS/EGS/BBB waves in Ontario, Canada. Antibodies were eluted from self-collected dried blood spots (DBS) tested by Enzyme linked Immunosorbent Assay for antibodies (IgG) against the receptor binding domain (RBD) and nucleocapsid proteins (NP). Values were converted to WHO BAU/ml International Standards. Monthly data captured vaccine boosters and breakthrough infections. The extended Cox proportional hazards models was used to investigate the association between the risk of breakthrough infection and predictor variables. RBD antibody levels were treated as a time-dependent covariate.

**Results:** 983/1286 participants submitted at least one DBS after the primary two dose vaccine series (Spring 2021). 538 participants submitted a DBS following the availability of bivalent BA.4/BA.5 vaccines (September 2022). Of this 175 (32%) developed their first breakthrough infection. In multivariable analysis of these 538 participants, breakthrough infection was not associated with the brand of the primary vaccine series (two mRNA2173- Moderna vs other brands or combinations) nor with underlying comorbidity (diabetes, cardiovascular disease, respiratory disease, cancer or transplant), current smoking, gender or non-white race. Those who were > 70 years of age were less likely to have breakthrough infection compared to those aged 30-50 years; HR 0.652 [0.434, 0.979]. Those with lower RBD antibody levels were not more likely to have breakthrough infection. The strongest correlation of protection was receipt of a bivalent vaccine booster, HR 0.363 [0.265, 0.496].

**Conclusion:** Bivalent booster vaccines matching the circulating COVID strains had the greatest association with protection against first breakthrough infection. We could not identify a threshold RBD antibody level for protection. The elderly were less likely to have breakthrough infection possibly related to behavioral differences.

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858 **Immunosuppressive Conditions Are Associated With Poor Outcomes in Patients Hospitalized for COVID-19**

Vijeeth Guggilla, Jennifer Pacheco, Alexandre Carvalho, Anna Pawlowski, Grant Whitmer, Chad Achenbach, Theresa Walunas

Northwestern University, Chicago, IL, USA

**Background:** Immunosuppressed adults (primary immunodeficiency (PI), HIV (unsuppressed and poor immune recovery), and solid organ transplant) are more likely to have severe COVID-19 and poor outcomes. Less is known about outcomes of severe COVID-19 for adults with specific immunosuppressive conditions compared to immunocompetent adults. We hypothesized that outcomes for adults with certain immunosuppressive conditions hospitalized for COVID-19 would be significantly worse.

**Methods:** We identified adults hospitalized for COVID-19 from 03/2020 to 05/2022 in EHR data from Northwestern Medicine (NM). Based on extracted diagnosis, lab, and procedure codes, we identified and categorized those with PI (antibody deficiency, combined immunodeficiency, common variable immunodeficiency, ataxia-telangiectasia, phagocyte deficiency, or complement deficiency); history of organ transplant (kidney, liver, heart, or lung), and HIV (defined above). Assessed COVID-19 outcomes included a seven-point ordinal severity scale based on maximum oxygen requirements during care (1 = Death), hospitalization length, and one-year cumulative incidence of death (all-cause).

**Results:** We included 10,713 adults hospitalized for COVID-19 at NM of which 214 had PI, 669 had history of organ transplant, and 183 had HIV (defined above). Patients with PI had a significantly higher mean hospitalization length of 11.8 days and a significantly lower mean maximum severity of 3.3 compared to 7.8 days and 3.9 in immunocompetent patients when controlling for obesity and T2DM. These effects were not significant when controlling for age. Patients with organ transplant had a significantly higher mean hospitalization length of 15.8 days when controlling for obesity, T2DM, and age, and a significantly lower mean maximum severity of 3.2 when controlling for obesity. Significantly higher mortalities were observed in PI and transplant (RR = 1.5, 95% CI 1.2 to 2.0; RR = 1.5, 95% CI 1.3 to 1.7), but these effects were not significant when controlling for age. There were no significant differences in any outcomes for patients with HIV.

**Conclusion:** Our results suggest patients with PI or organ transplant hospitalized for COVID-19 may have worse clinical outcomes compared to immunocompetent patients. This contrasts with patients with HIV, who had similar outcomes to immunocompetent patients, suggesting that people with different immunosuppressive phenotypes may respond variably to severe COVID-19.
860 COVID-19 Omicron Infection and Severe Outcomes in HIV and Matched Non-HIV Cohorts in Ontario, Canada
Ann N. Burchell1, Catharine Chambers1, Lena Nguyen1, Curtis L. Cooper1, Abigail E. Kroeh2, Hasina Samji3, Cecilia T. Costinuk3, Asian H. Anis4, Jeffrey C. Kwong5, Tahim Moinhedin6, Sarah A. Buchan7, Lawrence Mbuagbaw8, Claire E. Kendall9, Devan Nambiar4, for the COVAXHIV Team
1Unity Health Toronto, Toronto, Canada, 2University of Toronto, Toronto, Canada, 3ICES, Toronto, Canada, 4Ottawa Hospital Research Institute, Ottawa, Canada, 5Other Institution – Follow-up needed, N/A, 6Simon Fraser University, Burnaby, Canada, 7Research Institute of McGill University Health Centre, Montreal, Canada, 8CHR Canadian HIV Trials Network, Vancouver, Canada, 9Public Health Ontario, Ontario, Canada, 10McMaster University, Hamilton, Canada, 11University of Ottawa, Ottawa, Canada, 12Gay Men’s Sexual Health Alliance, Toronto, Canada

Background: There is concern that people living with HIV may be at greater risk for COVID-19 infection or severe outcomes, and that vaccination may offer less protection, compared to those not living with HIV. Our aim was to quantify rates of SARS-CoV-2 testing and COVID-19 infection and hospitalization/death in the Omicron period and compare this by HIV and vaccination status.

Methods: We identified community-dwelling people living with HIV aged ≥19 years in health administrative databases using a validated algorithm and followed them from January 2, 2022, to March 31, 2023, along with an HIV-negative cohort matched on age, sex, census tract, and immigrant status. Vaccination status was ascertained from the provincial COVID-19 vaccine registry. We report rates per 1000 person-years (PY) for time to first PCR testing for SARS-CoV-2, PCR-confirmed infection, and COVID-19-related hospitalization/death among HIV and non-HIV matched cohorts, with 95% confidence intervals (CI).

Results: A total of 20,978 people living with HIV and 20,978 matched HIV-negative individuals were included. Cohorts were comparable by matching factors age (mean 50.5 years), sex (21.9% female), region, and immigrant status (72.3% non-immigrant), and had a similar number of comorbidities. However, people living with HIV were more likely than HIV-negative individuals to have received 3+ doses of SARS-CoV-2 vaccine (31.5% cf 23.9%), recent influenza vaccine (42.9% cf 29.1%), and certain comorbidities: chronic kidney disease (5.9% cf 2.6%), frailty/dementia (5.3% cf 2.7%) or advanced liver disease (3.5% cf 1.2%). Rates of SARS-CoV-2 PCR testing and COVID-19 outcomes were consistently higher among people living with HIV, even when compared within vaccine dose strata (Table). Compared with HIV-negative hospitalized cases (n=100), hospitalized cases who were living with HIV (n=215) were more likely to be vaccinated (88.4% cf 79.0%); they were also younger, more likely to be female or an immigrant, and differed in comorbidities.

Conclusion: Timely booster doses and efforts to reduce SARS-CoV-2 exposure remain important for people living with HIV given higher rates of infection and severe outcomes. Limitations include incomplete confounding control and imprecision for severe events which were rare. Ongoing monitoring is needed for more recent vaccine formulations and against newer variants. The figure, table, or graphic for this abstract has been removed.

861 SARS-CoV-2 Infection and Hospitalization in Immunocompromised Children: A Population-Based Study
Costanza Di Chiara1, Arianna Giugni2, Marthe Le Prevost3, Elisa Barbieri4, Angela Lapугетлиц1, Carlo Giaquinto1, Danielle Donà1, Daniele Donà1, Marthe Le Prevost3, Costanza Di Chiara1, Arianna Giugni2, Marthe Le Prevost3, Elisa Barbieri4, Angela Lapугетлиц1, Carlo Giaquinto1, Danielle Donà1, Daniele Donà1
1University of Padova, Padova, Italy, 2University of Milano – Bicocca, Milan, Italy, 3ICU Great Ormond Street Institute of Child Health, London, United Kingdom, 4University of Oslo, Oslo, Norway

Background: The burden of SARS-CoV-2 infection in immunocompromised children remains unclear due to limited population-based studies. We aimed to assess the incidence of SARS-CoV-2 infection and hospitalization in children with and without immunocompromising conditions.

Methods: We conducted a population-based cohort study of children aged 0–14 years in the Veneto region, Italy, from February 2020–May 2023. Data were obtained from an Italian pediatric primary-care database (PediNet) linked to the Veneto region’s hospitalization and COVID-19 nasopharyngeal swab (NPS) registries. Three groups of children were included in this cohort: children with an immunocompromising condition and/or on immunosuppressive treatments (IC), non-immunocompromised children with at least one specific comorbidity other than immunocompromising diseases (non-IC), and healthy children (HC).

Results: A total of 20,978 people living with HIV and 20,978 matched HIV-negative individuals were included. Cohorts were comparable by matching factors age (mean 50.5 years), sex (21.9% female), region, and immigrant status (72.3% non-immigrant), and had a similar number of comorbidities. However, people living with HIV were more likely than HIV-negative individuals to have received 3+ doses of SARS-CoV-2 vaccine (31.5% cf 23.9%), recent influenza vaccine (42.9% cf 29.1%), and certain comorbidities: chronic kidney disease (5.9% cf 2.6%), frailty/dementia (5.3% cf 2.7%) or advanced liver disease (3.5% cf 1.2%). Rates of SARS-CoV-2 PCR testing and COVID-19 outcomes were consistently higher among people living with HIV, even when compared within vaccine dose strata (Table). Compared with HIV-negative hospitalized cases (n=100), hospitalized cases who were living with HIV (n=215) were more likely to be vaccinated (88.4% cf 79.0%); they were also younger, more likely to be female or an immigrant, and differed in comorbidities.

Conclusion: Timely booster doses and efforts to reduce SARS-CoV-2 exposure remain important for people living with HIV given higher rates of infection and severe outcomes. Limitations include incomplete confounding control and imprecision for severe events which were rare. Ongoing monitoring is needed for more recent vaccine formulations and against newer variants. The figure, table, or graphic for this abstract has been removed.
(IR) and 95% CI was considered within ten days after a positive NPS per 10,000 person days.

Results: In total, 26,606 children with active follow-up and at least one COVID-19 NPS within the study period were included in the analysis. Overall, 23,858 were HC, 2,527 non-IC, and 221 IC. Compared to HC, we observed the same risk of SARS-CoV-2 primary infection in IC (aHR = 0.92 [95% CI: 0.57-1.48]) and non-IC (aHR = 1.03 [95% CI: 0.91-1.21]) (Figure). Among 14,968 children with a positive NPS, IC had a higher IR of being hospitalized (IR = 4.97 [95% CI: 1.99-8.94]) compared to non-IC (IR = 2.72 [95% CI: 1.88-3.77]) and to HC (IR = 2.03 [95% CI:1.79-2.27]) (Figure). IC (aHR = 1.32 [95% CI:0.86-1.91]) and non-IC (aHR = 1.17 [95% CI:0.91-1.51]) were more likely to be vaccinated against COVID-19 than HC (Figure).

Conclusion: Similar SARS-CoV-2 infection likelihood and higher incidence of hospitalization were observed in IC compared to HC. Greater hospitalization rates in IC may be partly due to lower thresholds for hospital admission for these patients. Hospital-setting surveillance studies evaluating additional outcomes of severity, including intensive care admission and death, are needed to confirm our findings.

Risk of SARS-CoV-2 primary infection

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<td>0.92 (0.57-1.48)</td>
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Incidence rate of hospitalization

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COVID-19 vaccination uptake

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863 Epidemiology, Clinical Features, and Severity of COVID-19 in Immunocompromised Children in Canada

Costanza Di Chiara,1 Tilmann Schober,2 Daniel S. Farrar,2 Julie A. Bettinger,1 Joanne E. Embree,2 Scott J. Halperin,3 Tajeddin Jadayi,3 Kescha Kazmi,3 Rupeena Purewal,3 Manish Sadarangani,3 Laura Saude,4 Karina A. Top,3 Fatima Kakkar,3 Jesse Papenburg,5 Shaun K. Morris3

1Hospital for Sick Children, Toronto, Canada, 2McGill University, Montreal, Canada, 3University of British Columbia, Vancouver, Canada, 4University of Manitoba, Winnipeg, Canada, 5Dalhousie University, Halifax, Canada, 6University of Calgary, Calgary, Canada, 7Jim Pattison Children’s Hospital, Saskatoon, Canada, 8Centre Hospitalier Universitaire Sainte-Josine, Montreal, Canada

Background: The impact of immunocompromised states on pediatric COVID-19 and outcomes remains unclear. We aimed to evaluate clinical features and severity of SARS-CoV-2 infection in hospitalized children with and without immunocompromising conditions.

Methods: We conducted a national surveillance study of children <17 years hospitalized for COVID-19 from April 2020—May 2022. Data were captured through two surveillance programs The Canadian Pediatric Surveillance Program and the Canadian Immunization Monitoring Program, ACrIve which covers ~90% of all Canadian tertiary-care pediatric beds. Incidental SARS-CoV-2 positive cases were excluded. Immunocompromised children (IC) were defined as those with an immunocompromising condition and/or on immunosuppressive treatment(s). We included children with at least one comorbidity which was recognized in a study for the severe COVID-19 defined by the following: severe illness, death, or death due to lower thresholds for hospital admission for IC. Population-based studies are needed to confirm these findings.

862 Hospital Mortality During Different SARS-CoV-2 Variant Waves in the EuCARE Multinational Cohort

Pontus Hedberg1, Milosz Pancewicz2, Giulia Marchetti3, Tilmann Schober2,4,5, Jesse Papenburg2,6,7,8, Manish Sadarangani3, Alessandro Cozzi-Lepri1,9, Anders Sønnerborg10, Scott A. Halperin2, for EuCARE WP11

1Karolinska Institute, Stockholm, Sweden, 2Pomeranian Medical University, Szczecin, Poland, 3University of Milano–Bicocca, Milan, Italy, 4Heinrich Heine University Düsseldorf, Düsseldorf, Germany, 5Imperial College London, London, United Kingdom, 6Winnipeg Medical University, Winnipeg, Manitoba, Canada, 7Dalhousie University, Halifax, Canada, 8University of Calgary, Calgary, Canada, 9University of Rome Tor Vergata, Rome, Italy, 10Paul Ehrlich Institut, Langen, Germany, 11Kenya Medical Research Institute, Kilifi, Kenya

Background: Investigating outcomes of hospitalized patients with COVID-19 throughout the pandemic is crucial to understand the effects of SARS-CoV-2 variants, previous immunity, and healthcare interventions. We compared 28-day in-hospital mortality in adults hospitalized with COVID-19 caused by Wild-type, Alpha, Delta, or Omicron variants. Whether the difference in risk by variant might vary by age was also evaluated.

Methods: We conducted a multinational cohort study including patients hospitalized with COVID-19 from 9 countries (EuCARE hospitalized study). Patients >18 years, hospitalized any time from 2020-02-01 to 2022-10-15 with a SARS-CoV-2 positive test were included. Variant was classified based on sequenced viruses (if available) or from national public metadata. In-hospital mortality was compared using the cumulative incidence (CI) function and Fine-Gray subdistribution hazard models adjusted for age, sex, and comorbidities which are risk factors for severe COVID-19. Results were shown age-stratified since there was evidence that age was an effect measure modifier.

Results: We included 38,585 SARS-CoV-2 infected hospitalized patients (16,754 females and 21,831 males; 19,763 Wild-type, 6,387 Alpha, 3,640 Delta, and 8,795 Omicron. For in-hospital mortality, an interaction between age group and variant was observed (P=0.03), driven by the youngest group for whom smaller differences in mortality risk by variant were seen (Figure). In the older groups, the largest differences were observed between Omicron and the other variants. Among patients aged ≥70 years, the aSHR for Delta vs. Omicron was 2.06 (95% confidence interval 1.75-2.43). This estimate was 2.72 (2.38-3.12) for Alpha vs. Omicron, and 2.94 (2.64-3.27) for Wild-type vs. Omicron. Among unvaccinated patients, the aSHR was 1.33 (1.03-1.74) for Delta vs. Omicron, 1.61 (1.32-1.98) for Alpha vs. Omicron, and 1.69 (1.42-2.02) for Wild-type vs. Omicron. When comparing Omicron sublineages, the aSHR for the BA.1 sublineage was 2.04 (1.85-2.25) for Alpha vs. Omicron, and 2.94 (2.64-3.27) for Wild-type vs. Omicron. Overall, the cumulative incidence and hazard of in-hospital mortality rates decreased throughout the study period, particularly during the need for any respiratory support (11.6% vs 27.7%; p<0.001) and ICU admission (5.4% vs 17.1%; p<0.001) were less frequent among IC (Panel-A). In multivariable analyses, IC were less likely to have severe COVID-19 compared to non-IC (aRR=0.33 [95%CI:0.20-0.50]). Subcategories of immunocompromise, such as immunodeficiency (aRR=0.44 [95%CI:0.29-0.69]) and immunosuppression (aRR=0.28 [95%CI:0.15-0.51]) were individually also associated with a reduced likelihood of severe COVID-19. Compared to children with comorbidities other than immunocompromise, IC were also less likely to have severe COVID-19 (aRR=0.23 [95%CI:0.15-0.35]) (Panel-B).

Conclusion: Lower risk of severe COVID-19 was observed in hospitalized IC compared to non-IC and to those with other comorbidities, potentially in part due to lower thresholds for hospital admission for IC. Population-based studies are needed to confirm these findings.
Effects of Pitavastatin on COVID-19 Incidence and Seriousness Among People With HIV

Background: Among people with HIV (PWH), COVID-19 is common and potentially severe. We leveraged data from the Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) to assess effects of pitavastatin on COVID-19 outcomes (incidence and serious cases) among a global cohort of PWH. Statin therapy was a priori hypothesized to reduce serious COVID-19 by 35%, with 200 anticipated serious COVID-19 events.

Methods: COVID-19 data collection was implemented in REPRIEVE in April 2020 to capture all events from the start of 2020. COVID-19 was defined by positive test or clinical diagnosis; serious COVID-19 according to ICH definition, including life-threatening events or those resulting in hospitalization or death. Among participants in follow-up on January 1, 2020, Cox proportional hazards modeling was used to estimate the hazard ratio (HR) of COVID-19 (pitavastatin/placebo), stratified by global burden of disease region. Modification of statin effect following COVID-19 vaccination was evaluated via interaction with time-varying updated vaccination status.

Results: Among 6909 PWH, 32% were natal females and 41% were Black or African-American. Median age was 53 years and median 10-year ASCVD risk score 4.5%. HIV-1 viremia was suppressed in 87%, 30% had CD4 <500 cells/mm³, 23% BMI ≥30 kg/m², 45% hypertension, and 3% diabetes. Treatment groups were balanced on baseline characteristics. COVID-19 was reported in 1689 participants, including 117 serious cases (Figure 1). In a global cohort of PWH, COVID-19 was defined by positive test or clinical diagnosis; serious COVID-19 according to ICH definition, including life-threatening events or those resulting in hospitalization or death. Among participants in follow-up on January 1, 2020, Cox proportional hazards modeling was used to estimate the hazard ratio (HR) of COVID-19 (pitavastatin/placebo), stratified by global burden of disease region. Modification of statin effect following COVID-19 vaccination was evaluated via interaction with time-updated vaccination status.

Conclusion: A global cohort of PWH, statin therapy had no effect on COVID-19 incidence but showed potential to reduce the risk of serious COVID-19 prior to COVID-19 vaccination. Vaccination was protective for serious COVID-19. Additional research is needed to understand and harness potential mechanisms of protective statin effects on serious COVID-19.
ICU-Acquired Infections More Common in Patients With COVID-19 Than in Patients With Influenza
Josefine Beck-Frisi, Magnus Gisslen, Aylin Yilmaz, Anna Lindblom, Jonatan Oras
Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Background: During the covid-19 pandemic, intensive care unit-acquired infections (ICU-AI) were frequently diagnosed in critically ill patients. The primary aim of the study was to determine the impact of ICU-AI on mortality and ICU-stay in patients with covid-19 and influenza. The secondary aim was to compare the microbial pattern in patients with covid-19 vs influenza.

Methods: This is an observational study including (1) all patients 18 years and older treated with invasive mechanical ventilation (IMV) due to covid-19 at Sahlgrenska University Hospital (SU) January 2020–March 2022, and (2) all patients 18 years and older treated with IMV due to influenza at SU January 2015–May 2023. Data was collected from medical charts and the microbiology laboratory at SU. The definition of ICU-AI required both clinical criteria and a positive culture of a significant pathogen, according to ECDC’s standards. Frequencies were compared by Fisher’s exact test and continuous variables by t-test and Mann Whitney U test.

Results: A total of 480 participants were included in the final analysis, of whom 436 had covid-19. ICU-AI was confirmed in 190 patients (44%) with covid-19 and 7 patients (16%) with influenza. Ventilator-associated lower respiratory tract infection (VA-LRTI) was most common, being present in 149 patients (34%) with covid-19 and in 5 patients (11%) with influenza. The most common pathogens associated with VA-LRTI are presented in the figure below. Blood stream infections were relatively common in patients with covid-19 (n = 77, 18%) but were rare in influenza patients (n = 3, 7%). Gram-positive bacteria and candida were the most frequent findings in blood cultures in both groups. Corticosteroid treatment was associated with an increased risk of ICU-AI in patients with covid-19 (adjusted OR 2.955, 95% CI 1.263–3.475). Median (range) number of days in ICU for patients with an ICU-AI was 27 (4–103) and 12 (2–69) for patients without ICU-AI (p < 0.001). Having an ICU-AI was also associated with an increased risk of 90-day mortality (adjusted OR 1.794, 95% CI 1.134–2.838).

Conclusion: Secondary infections were more common in critically ill patients with covid-19 than with influenza and were associated with an increased time in ICU and mortality. Gram-negative bacteria caused a majority of VA-LRTI in patients with covid-19, while S. aureus was the singular most common pathogen in VA-LRTI in patients with influenza and covid-19.

867 Plasma Thrombomodulin Predicts Thrombotic Events & Mortality in Patients Hospitalized With COVID-19
Sergio Padilla1, Pascal Marco2, Ana Marco-Rica1, Christian Ledesma1, Carolina Ding1, Marta Fernandez-Gonzalez1, Alba de la Rica1, Javier Garcia-Arellano1, Paula Mascarell1, Angela Botella1, Nuria Ena2, Lidia Garcia1, Jose Carlos Asejon1, Mar Masia1, Felix Gutierrez1
Hospital General Universitario de Elche, Elche, Spain, Alzira University Hospital Universitario, Alicante, Spain

Background: Plasma concentration of soluble thrombomodulin (sTM) is a marker of endothelial damage, and its elevation has been linked to cardiovascular diseases. The study aims to evaluate the predictive potential of sTM for thrombotic events, one of the most serious complications of COVID-19.

Methods: Nested case-control study within a large cohort of hospitalized COVID-19 patients throughout the pandemic. Cases involved serious venous and arterial thrombotic events (TE) up to 28 days following hospital admission and they were compared with controls matched by sex, age, Charlson comorbidity index, and WHO-COVID-19 severity scale by propensity score (PS) in a 1:3 ratio. We determined the plasma concentration of sTM in all available frozen samples collected prior to the TE using an automated immunoassay technique.

Results: Between March 1st, 2020 and July 31st, 2022, a total of 2524 patients were hospitalized due to SARS-CoV-2 infection (22% Omicron variant). Forty-three percent of them were female and the median (Q1, Q3) age at admission was 67 (54, 80) years. There were 75 TE (58 venous-TE: 48 pulmonary embolism (PE) and 10 deep vein thrombosis (DVT); 17 arterial-TE (AT)) accounting for an incidence rate [95% CI] of 1.17 (0.92-1.47) per 1000 patient-days of follow-up. Frozen plasma samples were available in 43 cases (29 PE, 6 DVT, 8 AT) and in 176 PS-matched controls. There was no significant correlation between sTM and D-dimer (DD) (R=Pearson p-value +0.01 [0.85]). Elevated plasma concentration of sTM was significantly associated with both mortality (median [Q1, Q3], 3.2 [2.16, 4.65] vs. 1.58 [1.11, 2.73] ng/mL; p=0.001) and TE (2.77 [1.67, 4.01] vs. 1.52 [1.1, 2.65] ng/mL; p=0.001), while DD showed a specific association with TE (2.1 [0.83, 5.6] vs. 0.66 [0.4, 1.12] mcg/mL; p-value =0.001). The association with thrombotic events remained in adjusted models (OR [95%CI] per unit increase, 1.31 [1.03-1.68] for sTM; 1.11 [1.02-1.28] for DD). The adjusted regression model that included both variables (sTM and DD) improved significantly the predictive capacity of the same model without sTM (p-value=0.011; sensitivity 84% and specificity 32% for TE diagnosis).

Conclusion: Elevated soluble thrombomodulin levels were significantly associated with both thrombotic events and mortality in hospitalized COVID-19 patients. The measurements of thrombomodulin, along with D-dimer plasma levels, could enhance thrombotic risk assessment in this population.

868 Decline in Time to Recovery From Mild-Moderate COVID-19 in a Large Platform Placebo-Controlled Trial
Ahmad Mourad1, Yue Gao1, Thomas Stewart1, Adrian F. Hernandez2, Susanna Naggie3, Christopher J. Lindsell4, for the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV)-6 Study Group and Investigators
1Duke University School of Medicine, Durham, NC, USA, 2Vanderbilt University, Nashville, TN, USA, 3University of Virginia, Charlottesville, VA, USA

Background: Epidemiological data suggest that the duration of symptoms in patients with COVID-19 has decreased over the course of the pandemic. Robust estimates of the magnitude of change are not yet available. We estimated the change in time to recovery from COVID-19 among participants enrolled in the placebo arms of the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV)-6 platform, the largest ongoing randomized clinical trial evaluating treatment of outpatients with COVID-19.

Methods: Outpatients with mild to moderate COVID-19 were randomized to one of five placebo-controlled intervention groups between June 2021 and January 2023. Participants completed daily symptom reporting for at least 14 days or until symptom resolution. We estimated time to sustained recovery, defined as the third day of 3 consecutive days without symptoms, for participants receiving placebo only. Cox proportional hazards model was used to assess differences in time to sustained recovery over yearly quartiles during the study period.

Results: Among 6,708 total participants randomized, 2,435 received a matched placebo to one of the active drugs. Overall, the median age was 48 (IQR 39-58) years, and 61% were female. The median time to sustained recovery decreased from 15 (95% CI 14-17) days in Q3 of 2021 to 10 (95% CI 9-11) days in Q1 of 2023. Among 135 participants recruited in the 3rd quarter (Q3) of 2021, only 20 (14.8%) reported any vaccination for SARS-CoV-2. This increased to 429/486 (88.3%) by Q2 of 2022. Participants recruited later in the trial had quicker time to sustained recovery (p<0.001) (Figure 1). The most important predictors of recovery were baseline symptom severity, the duration of symptoms, sex, and calendar time of enrollment.

Conclusion: Time to recovery in outpatients with mild to moderate COVID-19 enrolled in the placebo arms of ACTIV-6 decreased over the duration of the trial. This trend is likely multifactorial including increased vaccination, prior infection, as well as evolving SARS-CoV-2 variants and subvariants. Future trials should take this into consideration when selecting endpoints and developing statistical analysis plans.
869 Estimating Optimal Anti-TB Drug Concentrations in a Prospective, Observational Cohort in Brazil

Gustavo Amorim¹, David W. Haas¹, Marina C. Figueiredo², Cody Staats³, Marcelo Cordeiro-Santos⁴, Afrânio L. Kritski⁴, Brian C. Hackey⁴, Bruno B. Andrade⁴, Timothy R. Sterling⁴, Valeria C. Rolla⁵, for the RePORT-Brazil Consortia
¹Vanderbilt University, Nashville, TN, USA, ²Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, Brazil, ³Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, ⁴Universidade de Sao Paulo, Sao Paulo, Brazil, ⁵Oswaldo Cruz Foundation - Fiocruz, Rio de Janeiro, Brazil

Background: Tuberculosis (TB) treatment is highly effective, but in real-world settings, >10% of TB patients experience drug toxicity or treatment failure. Current target drug levels of standard anti-TB medications (isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB)) are based on expected levels two hours after dosing. We assessed a large Brazilian cohort of pulmonary TB patients undergoing anti-TB therapy for concomitant optimal drug levels associated with minimal toxicity and maximum effectiveness.

Methods: Plasma drug levels from participants enrolled in Regional Prospective Observational Research in Tuberculosis (RePORT)-Brazil were used to estimate therapeutic drug range for all four anti-TB drugs. Pharmacokinetic (PK) analyses using drug concentrations and time since last anti-TB dose were performed using Extreme Gradient Boosting. The PK model was built using 10-fold cross-validation, adjusting for sex, BMI, HIV positivity, smoking and alcohol use, substance use, HbA1c, ancestry informative genetic markers, directly observed therapy assigned at baseline, and NAT2 acetylator status. Maximum drug concentrations (Cmax) computed from individual PK profiles were tested for correlation with TB treatment outcomes: toxicity (Grade ≥ 3 adverse events) and treatment failure or recurrence. Safety and effectiveness bounds were defined as drug concentrations that would lead to probabilities of toxicity or treatment failure/recurrence of no more than 5%, respectively. Confidence intervals were computed after 999 bootstrap resamples. Therapeutic drug range was defined as concentrations that were both safe and effective.

Results: There were 966 plasma samples from 459 participants. Overall, 11 (2.4%) experienced toxicity after month 1, and 13 (2.8%) had treatment failure or TB recurrence. Log-transformed Cmax for RIF was associated with greater odds of toxicity: Odds Ratio (OR) = 12.9 [95% CI=3.5-47.1]. Log-transformed Cmax for INH, EMB, and PZA were associated with decreased odds of failure/recurrence: OR = 0.5 [0.2-0.9], OR = 0.6 [0.4-1.0], and OR = 0.4 [0.2-0.8], respectively. Therapeutic drug ranges for all four anti-TB drugs are shown in the Table.

Conclusion: Our findings suggest target Cmax values, particularly for INH and RIF, that differ somewhat from currently recommended targets. Further studies with less variation in PK profiles are still needed to compute optimal targets for concentration ranges.

870 Pharmacogenetic Associations With HIV-1 Virologic Suppression Among Patients with TB/HIV in Brazil

Felipe Ridolfi¹, Gustavo Amorim², David W. Haas², Maria B. Arraial³, Cody Staats³, Marcelo Cordeiro-Santos³, Afrânio L. Kritski³, Marina C. Figueiredo³, Bruno B. Andrade³, Timothy R. Sterling³, Valeria C. Rolla³, for the RePORT-Brazil Consortia
¹Vanderbilt University, Nashville, TN, USA, ²Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, Brazil, ³Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, ⁴Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil

Background: Human genetic variants can affect TB and HIV drug pharmacokinetics (PK), which may affect risk for toxicity or treatment failure. Here we evaluated associations between pre-specified genetic variants and HIV virologic suppression (VS) among patients treated for TB and HIV.

Methods: We included TB/HIV RePORT-Brazil participants who initiated standard TB treatment (2 months of isoniazid/rifampicin (or rifabutin)/pyrazinamide/ethambutol, then 4 months or more of isoniazid/rifampicin (or rifabutin)), and who also received antiretroviral therapy (ART) during this period. The outcome was HIV-1 VS (for this study we considered as <50 HIV-1 RNA c/mL after at least 2 weeks of ART. Regimens were categorized as containing an integrase strand transfer inhibitor (INSTI) or non-nucleoside reverse transcriptase inhibitor (NNRTI). We genotyped UGT1A1*1 rs87188729 that affects doluglitazone (DTG) and raltegravir (RAL) PK, and CPY2B6 rs374724, rs28399499, rs4803419 that affect efavirenz (EFV) PK, all have defined normal, intermediate, and poor metabolizer groups. Genotyping was by MassARRAY iPLEX Gold. We compared outcome proportions (Fisher’s test) and time-to-VS (survival analysis, Wilcoxon-Gehan test).

Results: Among 194 TB/HIV participants included, 88 (45%) achieved VS. RAL was the most frequent INSTI (n=88, 88%), and EFV the most frequent NNRTI (n=76, 99%); and one participant used etravirine. In the INSTI group, similar proportions of VS were achieved for UGT1A1 normal (n=16, 39%) and intermediate (n=17, 41%) genotypes. Among participants receiving EFV, those who achieved VS were more likely to be CYP2B6 intermediate metabolizers (n=23, 70%). There were inconclusive associations comparing the proportions of VS among INSTI- and EFV-based ART, and based on CPY2B6 and UGT1A1 genotypes (Table 1). Furthermore, no consistent associations were found comparing the time-to-VS among ART regimens and genotypes.

Conclusion: In this cohort of patients treated for TB/HIV, genetic variants that affect ART PK were not significantly associated with likelihood of VS.

Table 1: ART and genotype groups stratified by HIV-1 Virologic Suppression

<table>
<thead>
<tr>
<th>ART group/Genotype</th>
<th>UGT1A1*1 rs87188729</th>
<th>CPY2B6 rs374724</th>
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<tr>
<td>Normal</td>
<td>12.9 (5.9-28.6)</td>
<td>9.0 (6.0-13.9)</td>
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<tr>
<td>Intermediate</td>
<td>17.5 (9.0-34.4)</td>
<td>11.7 (7.5-19.4)</td>
</tr>
<tr>
<td>Poor</td>
<td>23.6 (13.3-41.9)</td>
<td>14.0 (8.6-22.6)</td>
</tr>
<tr>
<td>Total</td>
<td>11.3 (8.0-16.0)</td>
<td>12.1 (9.0-16.0)</td>
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</table>

*Results for UGT1A1 are not significantly different across effect and reference (OR = 1.0); **Results for CPY2B6 are not significantly different across effect and reference (OR = 1.0)
formation. Here we describe the PK of INH, INA, AcINH, AcHz, and Hz in AP and PP WWH receiving IPT.

Methods: Samples and data from intensive PK assessments among WWH enrolled in P1078 were included in the analysis. WWH received INH 300 mg once daily for at least 2 weeks prior to the intensive PK assessment. Samples were collected at 0, 1, 2, 4, 6, and 8 h post-dose. Intensive PK during AP occurred ≥28 weeks of gestation and 16 (±2) weeks PP. INH, INA, AcINH, AcHz, and Hz were quantified using a validated LC-MS/MS method (lower limit of quantification 10 ng/mL for all analytes). PK data were analyzed using noncompartmental methods. Data were summarized descriptively by pregnancy stage and NAT2 acetylation status (fast, intermediate, slow). Linear mixed models were used to compare percent differences (95% confidence intervals (CI)) between AP vs. PP for each analyte.

Results: Data from 31 WWH were analyzed (10 AP and PP, 4 AP only, 17 PP only). The median (range) gestational age at entry was 26 (14-34) weeks. Maternal median (range) age was 29 (18-41) years with 81% Black and 19% Asian. Observed AcINH exposures were higher among intermediate and fast acetylators, whereas INH, INA, and AcHz exposures were higher in intermediate and/or slow acetylators (Table). INA and AcHz were lower AP versus PP after controlling for NAT2 status. Hz was quantifiable in 5/14 AP and 23/27 PP WWH, with numerically higher AUCs observed PP.

Conclusion: These data demonstrate relationships between NAT2 and the formation of INH metabolites in pregnant and postpartum WWH. INA and AcHz AUCs were higher and Hz was detectable in more women during PP. Analyses to examine the influence of pregnancy and pregnancy effect on the formation of INH metabolite PK in the overall study population and potential associations with hepatopathy in P1078 are ongoing.

Table. Influence of Pregnancy and NAT2 Status on INH and Metabolite AUCs

| Analyte | AP AUC (ng/mL h) | INH | AcINH | AcHz | Hz | PP AUC (ng/mL h) | INH | AcINH | AcHz | Hz
<table>
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</thead>
<tbody>
<tr>
<td>NAT2 Fast</td>
<td>Intermediate &amp; Slow</td>
<td></td>
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<td></td>
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<tr>
<td>INH</td>
<td>21.9</td>
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<td>AcINH</td>
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<td>68.4</td>
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<td>40.9</td>
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<td>NAT2 Slow</td>
<td>Intermediate &amp; Slow</td>
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</tr>
<tr>
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<td>12.1</td>
<td>10.3</td>
<td>9.2</td>
<td>12.1</td>
<td>10.3</td>
<td>9.2</td>
<td>12.1</td>
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<td>12.1</td>
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<td>4.7</td>
<td>3.6</td>
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<td>4.7</td>
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<tr>
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<td>6.4</td>
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873 Cardiac Involvement in Tuberculosis Patients at the Start and End of Treatment in Southern Africa

Daryoush Samimi1, Guy Moudal, Douglas Chibombo2, Sibale Xulu2, Nicolas Banholzer3, Stefano De Marchi4, Gunar Gunther4, Denise Evans5, Carolyn Bolton5, Matthias Egger6, Thomas Pilgrim7, Lukas Fenner6, for ILEDA Southern Africa (ileDA-SA)
1University Hospital of Bern, Bern, Switzerland; 2Centre for Infectious Disease Research in Zambia, Lusaka, Zambia; 3University Teaching Hospital, Lusaka, Zambia; 4Health Economics and Epidemiology Research Office, Johannesburg, South Africa; 5Institute of Social and Preventive Medicine, Bern, Switzerland; 6Center for Infectious Disease Research in Zambia, Lusaka, Zambia

Background: Tuberculosis (TB) primarily affects the lungs but can also involve cardiovascular structures such as the pericardium. Little is known about the type, frequency, and clinical significance of cardiovascular involvement in people with TB. We established two cohorts in Zambia and South Africa to measure pulmonary and cardiovascular complications in HIV-positive and HIV-negative TB patients before and after TB treatment.

Methods: As part of the ongoing TB cohort, we consecutively recruited clinically or microbiologically confirmed TB patients (>15 years old) between October 2022 and July 2023 in Lusaka/Zambia and Johannesburg/South Africa. Clinical and laboratory data were collected electronically from all participants. We performed standardized transthoracic echocardiography at the start (baseline) and at the end of TB treatment (6-month follow-up). We estimated associations of pericardial changes with baseline characteristics using linear regression models.

Results: We included 240 TB patients; 50 had follow-up images. The median age was 34 years (Interquartile range: 28–42 years); 187 (78%) were men, and 92 (38%) were HIV-positive. At baseline, most frequent echocardiographic abnormalities were pericardial effusion (PE; 116 persons, 48%) and pericardial thickening (PT; 82, 34%), followed by left atrial (LA) dilation (39/225, 17%) and pericardial calcifications (7, 3%); 4/49 (8%) had diastolic dysfunction. Signs of constriction (SoC) were observed in 94/201 (47%) patients and a definitive diagnosis of constriction was made in 15/70 (21%) patients at baseline. Abnormal LV geometry was found in 111/228 (49%) patients, most commonly concentric remodeling (97, 87%). LV dilatation was seen in 2/225 (1%) patients and RV dilatation in 14/208 (7%). Left ventricular (LV) systolic function was preserved in almost all patients (235/237, 99%), and right ventricular (RV) systolic dysfunction was observed in 11/224 (5%) patients. PT tended to be negatively associated with HIV infection, PE with younger age (Figure A). In 50 patients with follow-up imaging, pericardial changes tended to improve during TB treatment (Figure B).

Conclusion: Cardiac involvement of TB patients at the time of treatment start were relatively frequent, particularly signs of restrictive pericarditis. Pericardial thickening tended to be less frequent in TB patients with HIV. Outcomes tended to improve during treatment, but long-term outcomes improved gonadal status, consistent with improved spermatogenesis. Body weight increased, indicating improved health status. Sputum cultures were negative in all participants from Week 8 onwards. Of 26 enrolled participants, 2 discontinued treatment due to elevated liver enzymes, one due to pyrazinamide resistance and one withdrew consent. Two participants experienced serious adverse events (infectious exacerbation of bronchiectasis and increased liver enzymes).

Conclusion: This is the first study designed to investigate severe testicular toxicity in adult males with DR-TB. Results show that pretomanid, as part of the BPaMZ regimen, does not appear to have negative effects on reproductive function in adult males with DR-TB.
such as heart failure and constriction need to be studied beyond the end of TB treatment.

Figure. Percentile distribution (PD), percentile thickness (PT), and signs of constriction in tuberculosis patients in South Africa and Zambia.

874 Efficacy and Safety of endTB Regimens for Fluoroquinolone-Susceptible RR-TB in People With HIV

Gustavo E. Velásquez1, M Goulliou2, E Berikova3, M Bonnet4, N Lachenal5, L Lecca6, L Oyewusi7, Michael L. Rich8, Nasseem Salahuddin9, Kwonjune J. Seung2, Sean Wasserman9, Francis Varaine10, Lorenzo Guglielmetti11, Carole D. Mitnick10, for the endTB Clinical Trial Group.

University of California San Francisco, San Francisco, CA, USA; Epicentre, Paris, France; Partners In Health, Astana, Kazakhstan; Université de Montpellier, Montpellier, France; ‘Hôpitaux Universitaires de Strasbourg’ – France; Pasteur Institute of Social and Preventive Medicine, Bern, Switzerland; Geneva, Switzerland; Socios en Salud Sursalud Peru CRIS, Lima, Peru; Partners In Health, Maseru, Lesotho, MA, USA; Indus Hospital, Karachi, Pakistan; ‘St. George’s University of London, London, United Kingdom; ‘Hôpitaux Universitaires de Strasbourg’ – France, Paris, France; Harvard Medical School, Boston, MA, USA.

Background: endTB (NCT012754765) was an open-label Phase 3 randomized, controlled clinical trial to evaluate the efficacy and safety of five 9-month, all-oral regimens for fluoroquinolone-susceptible rifampin-resistant TB, compared to the WHO-recommended standard of care, in people 15 years of age or older. In the primary analysis, three experimental regimens (9BLMZ, 9BCLLfxZ, and 9BDLLfxZ) had noninferior efficacy compared to the control and compared to the WHO-recommended standard of care, in people 15 years of age or older. In the primary analysis, three experimental regimens (9BLMZ, 9BCLLfxZ, and 9BDLLfxZ) had noninferior efficacy compared to the control and were safe. Here, we present efficacy and safety results among people with HIV (PWH).

Methods: endTB inclusion was irrespective of HIV status or CD4 count. The safety population included all randomized participants who started study treatment, and the modified intention-to-treat (mITT) population included those in the safety population who had a positive pre-randomization TB culture and no resistance to study drugs. The primary efficacy endpoint was favorable outcome at Week 73 post-randomization, defined as either (1) two consecutive, negative cultures (one between Weeks 65 and 73); or (2) two favorable evolution. Unfavorable outcomes included death, treatment failure, drug addition/replacement, and retreatment. Safety outcomes were Grade 3-4 adverse events (AEs); serious AEs (SAEs); deaths; AEs leading to permanent discontinuation of all study drugs. We estimated changes in the QoL physical (QoL-PCS) and mental (QoL-MCS) components and the short form health status (SF-12) scores.

Results: From 2017-2021, we randomized 754 participants in 7 countries; 104 (13.8%) were PWH. The safety population included 103 (99.0%) and the mITT 98 (94.2%). Median age was 39 years (range 19-70 years); 46 (46.9%) were female; median CD4 count was 296 (range 5-1294); and 61 (62.2%) were on antiretrovirals at baseline. Two of the noninferior experimental arms from the endTB trial demonstrated high efficacy in PWH, with favorable outcomes in 93.3% (9BLMZ) and 100.0% (9BCLLfxZ). The remaining three experimental arms and the control also performed well but showed relatively lower efficacy: 70.6% (9BDLLfxZ), 83.3% (9DCLLfxZ), 73.3% (9DCMZ), and 89.5% (control). Grade 3-4 AEs, SAEs, AESIs, and AEs leading to drug discontinuation were more common in the control than in experimental arms.

Conclusion: Among regimens that were noninferior to the WHO control in the primary analysis, 9BLMZ and 9BCLLfxZ appeared to be particularly efficacious and safe for PWH. Additional research is needed to establish optimal all-oral shorter regimens in PWH.

875 Changes in Physical and Mental Health Among TB Patients During Treatment in Southern Africa

Nicolas Banholzer1, Guy Musa2, Denise Evans3, Jacqueline Huwa4, Idiovino Rafael2, Cordelia Kunzekwonyika5, Balliit Marie Balli6, Gunar Gunther7, Matthias Egger7, Lukas Fenner2, for IeDEA Southern Africa (IeDEA-SA)

1Institute of Social and Preventive Medicine, Bern, Switzerland; 2Switzerland, Geneva, Switzerland; 3Center for Infectious Disease Research in Zambia, Lusaka, Zambia; 4Health Economics and Epidemiology Research Office, Johannesburg, South Africa; 5‘Lighthouse Trust Clinic, Lilongwe, Malawi; 6SolidarMed, Luzern, Switzerland; 7University Hospital of Bern, Bern, Switzerland.

Background: Tuberculosis (TB) contributes to high morbidity and mortality worldwide. TB may also impaire people’s quality of life (QoL) and physical fitness. We studied the change in health-related QoL and functional exercise capacity (cardio-pulmonary function) in TB patients living with and without HIV between treatment start and end.

Methods: We recruited people with TB aged ≥15 years between October 2022 and July 2023 in five ongoing cohorts in Zambia, South Africa, Malawi, Mozambique, and Zimbabwe. At the start (baseline) and end of TB treatment, we measured physical and mental health outcomes using the standardized QoL Short Form Health Survey (SF-12), depression using the Patient Health Questionnaire (PHQ-9), and physical fitness using the 6-min walk test (6MWT). We also collected age, sex, HIV status, type of diagnosis (clinical vs microbiologically confirmed), chest X-ray findings, and TB multidrug resistance. We estimated changes in the QoL physical (QoL-PCS) and mental (QoL-MCS) t-score component, the depression score (PHQ9-S), and 6MWT distance (6MWT-D) between the start and end of TB treatment. We estimated the association of these changes with baseline characteristics using Bayesian multivariable log-linear regression models and cohort-specific random effects.

Results: We included 200 TB patients with at least one outcome at the start and end of treatment. Overall, the median age was 36 years (Interquartile Range [IQR]: 28-43 years), 55 (27%) female, 79 (40%) living with HIV, 101 (51%) were clinically diagnosed, 34 (17%) had lung cavitations, and 3 (1%) presented with TB drug resistance. At treatment start, overall median QoL-PCS was 37 (IQR: 28-43), QoL-MCS was 44 (IQR: 39-50), PHQ9-C was 6 (IQR: 3-10), and 6MWT-D was 400m (IQR 332-464). QoL-PCS increased by 39% (95%-Credible Interval [CI] 29-43), QoL-MCS was 44 (IQR 39-50), PHQ9-CS was 6 (IQR 3-10), and 6MWT distance (6MWT-D) increased from 347 (IQR: 291-409) to 400m (IQR 332-464) at the end of treatment. We estimated changes in physical and mental capacity (cardio-pulmonary function) in TB patients living with and without HIV between treatment start and end.

Conclusion: QoL and physical fitness were reduced at the start of treatment in South African TB patients but improved by the end of treatment. More emphasis should be placed on improving clinical management with respect to QoL and mental health aspects during and after TB treatment.
876 Cryptococcal Meningitis as an Indicator for Monitoring HIV Treatment Program Success in Botswana
James Milburn, Dokedtie Ntwayageh, Rachita Suresh, Ketabshiabile Hngal, Tony Chebani, Tsehgo B. Leeme, David S. Lawrence, Daniel Grift, Mark W. Tenforde, Ava Avalos, Dinah Ramaabya, Justus Ogando, Margaret Mokonene, Madisa Mine, Joseph N. Jarvis
1 London School of Hygiene & Tropical Medicine, London, United Kingdom, Botswana–University of Maryland School of Medicine Health Initiative, Gaborone, Botswana, 2 Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 3 Botswana Ministry of Health, Gaborone, Botswana, 4 Botswana–Uphenn Partnership, Gaborone, Botswana, 5 Clinton Health Access Initiative, Nairobi, Kenya, 6 University of Botswana, Gaborone, Botswana, 7 National Health Laboratory, Gaborone, Botswana

Background: Cryptococcal meningitis (CM) remains a frequent opportunistic infection among individuals living with advanced HIV disease (AHD) in much of Africa. Despite widespread expansion of ART programmes, modelled estimates indicate that the incidence of CM has not substantially decreased between 2014 and 2020, suggesting that the prevalence of AHD remains high in the region. CM incidence could provide a useful indicator for monitoring AHD rates and HIV program success, especially in the context of declining access to CD4 testing; most patients present to healthcare facilities with a well-defined clinical syndrome, and diagnosis can be made using low-cost and highly accurate rapid diagnostic tests. Very few countries collect reliable statistics on CM incidence, and the impact of WHO universal HIV treatment guidelines on the incidence of AHD is not known.

Methods: We analyzed 8 years of national meningitis surveillance data (2015-2022) captured from electronic health records in Botswana. All laboratory records from cerebrospinal fluid samples analysed within government healthcare facilities in Botswana were extracted from a central online repository. Adjustments for missing data were made based on triangulation for underestimates using comprehensive prospective datasets. CM case frequency was enumerated using a case definition and incidence was calculated using national census data.

Results: A total of 1,744 episodes of CM were identified. The estimated national incidence of CM in Botswana approximately halved between 2015 and 2022, from 15.0 cases/100,000 person years (PYO) (95% CI 13.5-16.7 cases/100,000) to 7.43 cases/100,000 PYO (95% CI 6.4-8.6 cases/100,000). Among all people living with HIV, the incidence of CM decreased from 92.0 cases/100,000 PYO (95% CI 82.2-102.6 cases/100,000 PYO) to 49.1 cases/100,000 PYO (95% CI 42.2-57.0 cases/100,000 PYO) between 2015 and 2022. There was no clear increase in the incidence of CM from 2015 to 2022. The highest incidence was observed in men and individuals aged 40-44. The proportion of cases diagnosed through rapid cryptococcal antigen (CrAg) testing increased during the study period from 35.5% to 86.3%.

Conclusion: CM incidence has decreased with expanded ART treatment but persists at a relatively high rate despite excellent reported ART coverage. Most cases are now diagnosed through the rapid CrAg lateral flow assay highlighting the potential of using CM as key indicator for programme success in the Treat All era.

Figure 1 Incidence of cryptococcal meningitis in Botswana/100,000 person years of observation from 2013-2022 (bar chart), overall incidence and 95% confidence intervals. UNAIDS estimate of total numbers of people living with HIV (black line) and number of people receiving ART (dotted line).

877 Effect of Smoking on Longitudinal Interferon-γ Release Assay Results Among Tuberculosis Contacts
Maria B. Arriaga, Gustavo Amorim
Vanderbilt University, Nashville, TN, USA

Background: Diagnosis of M. tuberculosis (Mtbc) infection in close tuberculosis (TB) contacts is critical for TB control. Interferon-gamma release assays (IGRAs) diagnose Mtbc infection, but the test is limited by assay variability, including the conversion (negative to positive) and reversion (positive to negative) of IGRAs responses that may not always reflect changes in Mtbc infection status. Several studies have revealed that smoking is a risk factor for Mtbc infection and TB disease but its effect on longitudinal IGRA results remains unknown.

Methods: We conducted a multi-site prospective study in RepORT-Brazil between 2015-2019 among close contacts of adults with culture-confirmed pulmonary TB. IGRA testing was performed at baseline, month 6 and month 24-30 after enrollment. IGRA results were categorized as IGRA-positive (maintained from baseline to last visit), IGRA-conversion (from negative to positive at any time), IGRA-reversion (from positive to negative at any time), and IGRA-negative (maintained from baseline to last visit). Associations between IGRA results and smoking status at baseline (current/former vs never) in contacts were evaluated using propensity score-adjusted logistic regression models to avoid overfitting. More specifically, we first estimated the propensity of smoking using Lasso. Next, we estimated propensity score was used as a covariate in the main outcome model, which regressed the outcome (IGRA-positive, IGRA-conversion, IGRA-reversion) on smoking status.

Results: There were 430 close contacts of 139 TB cases. Of the contacts, 89 (21%) were IGRA-positive, 30 (7%) were converters, and 30 (7%) were reverts; 22 (5.1%) had an indeterminate result. The frequency of smoking among contacts was assessed at 26 (29.2%), 7 (23.3%) and 3 (10%) in IGRA-positive, IGRA-conversion and IGRA-reversion groups, correspondingly. Smoking in contacts was associated with lower odds for IGRA-reversion (adjusted odds ratio=0.09; 95% confidence interval=[0.01,0.73]). We did not detect associations between smoking and IGRA-positive or IGRA-conversion at the 5% level.

Conclusion: Contacts who reported smoking (past/former) had lower odds of reverting from an IGRA-positive to an IGRA-negative result. Our findings highlight the importance of smoking on longitudinal IGRA results. This has implications for clinical care and clinical trials in which IGRA status is monitored or used as an outcome, such as TB vaccine trials.

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</tr>
<tr>
<td>Smoking (contact)</td>
<td>1.96 (0.94-4.03)</td>
<td>0.279</td>
</tr>
<tr>
<td>Propensity score</td>
<td>0.98 (0.84-1.17)</td>
<td>0.422</td>
</tr>
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</table>

878 Viral Suppression Among Adults Receiving Dolutegravir-Based ART and 3-Months Isoniazid-Rifapentine
Leila H. Chaisson, Shafic Makumbi, David Dowdy, Carina Marquez, Derek T. Armstrong, Bishop Dripa, Patrick P. Phillips, Fred C. Semitala, Christina Yoon
1 University of Illinois at Chicago, Chicago, IL, USA, 2 Infectious Diseases Research Collaboration, Kampala, Uganda, 3 The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 4 University of California San Francisco, San Francisco, CA, USA, 5 The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 6 Makerere University College of Health Sciences, Kampala, Uganda

Background: Prior studies have reported conflicting results regarding the safety and/or effectiveness of rifapentine-based tuberculosis (TB) preventive therapy with dolutegravir (DTG)-based ART. In an interim analysis, we previously found that co-administration of 3HP (3 months weekly isoniazid+rifapentine) with TDF/3TC/DTG (TLD) was safe for adults initiating routine ART, but that those who received 3HP+TLD were less likely to achieve 6-month viral suppression compared to those who received TLD alone. Here, we present an updated analysis of 1) safety of 3HP+TLD and 2) viral suppression among those initiating 3HP+TLD vs TLD alone.

Methods: In an ongoing phase 3 randomized trial comparing two TB screening strategies among adults with HIV initiating routine ART in Uganda (NCT04557176), participants who screened negative for TB were assessed for

- "3-Months Isoniazid-Rifapentine"
- "3HP+TLD vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
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- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentine" vs TLD alone."
- "3-Months Isoniazid-Rifapentenc..."
3HP eligibility using a standardized questionnaire and liver enzyme testing; those eligible initiated self-administered 3HP two weeks after ART initiation. HIV viral load (VL) was measured as part of routine care at 6 and 12 months. We evaluated 3HP discontinuation due to drug toxicity among participants receiving 3HP+TLD, and compared viral suppression (VL ≤ 50 copies/mL) among adults who initiated 3HP+TLD vs TLD alone using chi-square tests and log-binomial regression.

**Results:** From November 2020 to January 2023, 1,379 participants without TB initiated 3HP, including 539 (39.1%) who initiated 3HP (163 participants receiving 3HP+TLD were previously reported). Those who initiated 3HP+TLD were more likely to be male (35% vs 31%, p = 0.06) and had higher pre-ART CD4 counts (median 296 vs 238 cells/µL, p < 0.01) than those who initiated TLD alone. Overall, 509 of 539 (94%) participants completed 3HP; reasons for discontinuation included adverse events (n = 2), TB diagnosis (n = 3), pregnancy (n = 3), and self-discontinuation (n = 22). Of 597 (71%) participants with 6-month VL results and 536 (39%) participants with 12-month VL results, there was no difference in viral suppression (VL ≤ 50 copies/mL) between those who received 3HP+TLD vs TLD alone (6-months: 72.7% vs 72.8%; adjusted risk ratio [ARR] 1.00, 95% CI 0.93-1.08; 12-months: 76.3% vs 77.3%; ARR 0.97, 95% CI 0.89-1.07).

**Conclusion:** Co-administration of 3HP+TLD was well-tolerated, with high completion. Now with data from 1,379 participants, and contrary to our interim analysis, there was no difference in viral suppression (VL ≤ 50 copies/mL) between those receiving 3HP+3HP vs TLD versus TLD alone.

**Outcomes of Adults With HIV Receiving TB Preventive Therapy in Kampala, Uganda**


*University of Illinois at Chicago, Chicago, IL, USA, Makerere University College of Health Sciences, Kampala, Uganda, Infectious Diseases Research Collaboration, Kampala, Uganda, University of California San Francisco, San Francisco, CA, USA, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA*

**Background:** After decades of underuse, tuberculosis preventive therapy (TPT) underwent rapid scale-up in Uganda in 2020 as part of a national campaign. This campaign overlapped with a pragmatic trial comparing the impact of TB screening strategies on TPT uptake among antiretroviral therapy (ART)-naive adults who also included standardized protocols for assessing TPT eligibility and TPT-specific follow-up. We compared clinical outcomes among trial participants who received study- vs clinic-initiated TPT.

**Methods:** In an ongoing randomized controlled trial (NCT04557176), participants who screened negative for TB were assessed for 3HP (3-months weekly isoniazid+rifapentine) eligibility using a standardized questionnaire and liver enzyme testing. Eligible participants self-administered 3HP two weeks after ART initiation; participants were assessed monthly for adverse events (AEs) using standardized checklists and adherence by pill count. Those ineligible for study-initiated 3HP were reassessed through routine care in the clinic (0.2% vs 2.1%, p = 0.01). We evaluated 3HP discontinuation due to drug toxicity among participants receiving 3HP+TLD, and compared viral suppression (VL ≤ 50 copies/mL) among adults who initiated 3HP+TLD vs TLD alone using chi-square tests and log-binomial regression.

**Results:** Of 1,719 total participants, 541 (31%) received study-initiated 3HP and 235 (14%) received clinic-initiated TPT (94% isoniazid; 6% 3HP). Median follow-up was 584 days (IQR 422-727). Study-initiated participants were younger (median 29 vs 32 years, p < 0.01), more often female (65% vs 59%, p < 0.01), and had higher pre-ART CD4 counts (median 295 vs 138 cells/µL, p < 0.01) than clinic-initiated participants. Permanent TPT discontinuations due to suspected AEs were uncommon for both groups (≤1.5%), but completion was higher for study- than clinic-initiated TPT (94.5% vs 76.4%, p < 0.01; Table). TB incidence was lower among participants initiating TPT through the study than through the clinic (0.2% vs 2.1%, p = 0.01).

**Conclusion:** Participants initiating 3HP through a randomized trial had higher CD4 counts, were more likely to complete TPT and had lower TB incidence than those who received TPT through routine clinic procedures. Standardized protocols to assess TPT eligibility, monitor AEs and ensure completion will be critical to optimizing the impact of TPT among people initiating ART in high TB/ HIV burden settings.

**Table. Outcomes among participants with HIV receiving study- vs clinic-initiated TPT**

<table>
<thead>
<tr>
<th>Study-initiated TPT</th>
<th>Clinic-initiated TPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median time to TPT start, days (IQR)</td>
<td>14 (14-15)</td>
</tr>
<tr>
<td>TPT discontinued due to AE</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>Completed TPT</td>
<td>511 (94.5%)</td>
</tr>
<tr>
<td>Incident TB</td>
<td>1 (0.2%)</td>
</tr>
</tbody>
</table>

**Abbreviations:** TPT (tuberculosis preventive therapy); IQR, interquartile range; AE, adverse event; TB, tuberculosis; Legend: TPT completion was assessed through recall; data on completion were missing for 42 clinic-initiated participants. *Incident TB among study-initiated participants diagnosed by Xpert MTB/RIF (1) and clinic-initiated participants by Xpert MTB/RIF (4) and culture only (1) at all participants with incident TB reported completing TPT.

**880 Long-Acting Injectable Rifapentine With Activity in a Mouse Model of Tuberculosis Preventive Therapy**

Henry Pertinez, Nicole C. Ammerman, Si Yang Li, Jonathan Massam, James J. Hobson, Alison C. Savage, Joanne Sharp, Joanne Hernott, Editja Kjikja, Eduardo Gallardo-Toledo, Megan Neary, Steve Rammard, Susan Swindells, Andrew Owen, Eric Nuermberger

*University of Liverpool, Liverpool, United Kingdom, The Johns Hopkins University, Baltimore, MD, USA, University of Nebraska Medical Center, Omaha, NE, USA*

**Background:** Use of long-acting injectables (LAIs) has the potential to simplify tuberculosis preventive therapy (TPT) addressing issues such as pill burden and adherence. Rifapentine (RPT) is a key component of shorter TPT regimens and has physicochemical and pharmacokinetic (PK) properties amenable to LAI formulation. The aim of this work was to characterize preclinical performance of a new RPT single phase spray dried nanosuspension LAI for use in TPT.

**Methods:** Single intramuscular dose PK profiles were first characterized in mice and rats. Based on mouse PK, 8 RPT-LAI regimens were used to evaluate bactericidal activity in a validated mouse model of TPT. RPT (93.75, 187.5, and 375 mg/kg) was administered via 1, 2 or 4 injections over 4 weeks in expectation of clearing a 0.6 µg/mL plasma target. With 3 control groups [untreated negative control: positive control daily oral isoniazid and RPT (1HP); 4 weeks of oral RPT], a total of 186 adult female BALB/c mice were used. Lung bacterial colony-forming units (CFU) counts and plasma RPT exposures were measured 2-8 weeks after the start of treatment. Group mean CFU counts were analyzed using 1-way ANOVA and plasma exposure with compartmental PK modeling.

**Results:** LAI-RPT demonstrated dose-linear PK for single injection doses of 187.5 and 375 mg/kg (AUC0-146 6629 and 13071 µg·h/mL, respectively) in mice, with PK disposition parameter estimates in keeping with reported mouse RPT values and a release-dependent, “flip-flop” terminal phase (Fig.1A). As for other successful LAI which demonstrate longer half-lives in larger species, longer exposure durations were observed in rats. All RPT-LAI regimens had bactericidal activity in mice, which was dose-dependent and greatest when divided into 4 weekly injections. Several regimens had bactericidal activity equal to or greater than the 1HP control regimen: 37.5 mg/kg x 1, 93.75 mg/kg x 2, 187.5 mg/kg x 2, 93.75 mg/kg x 4, and 46.9 mg/kg x 4 injections (Fig.1B). PK after 2nd, 3rd or 4th dose in multiple injection regimens were inconsistent with single injection PK, with plasma exposures falling below 0.6 µg/mL target by 1 week post dose.

**Conclusion:** These data provide proof-of-concept for RPT-LAI to achieve efficacy comparable to 1HP in a validated mouse TPT model. Cross-species PK data suggest that efficacious RPT exposures should be achievable in humans. Further work to characterize the impact of repeat dosing on PK and conduct GLP toxicology studies to support first in human evaluation are underway.

**Figure 1. A: Mouse Bacterial Clearance With Activity in a Mouse Model of Tuberculosis Preventive Therapy**
One-Dose Efficacy of Long-Acting Injectable Diarylquinoline in Mouse Model of TB Preventive Therapy

James J. Hobson1, Si Yang Li1, Nicole C. Ammerman1, Jonathan Massam1, Joanne Sharp1, Nader Fotouhi2, Steve Rennard3, Andrew Owen4, Eric Nuernberger5
1University of Liverpool, Liverpool, United Kingdom, 2The Johns Hopkins University, Baltimore, MD, USA, 3AbbVie, 4Global Alliance for TB Drug Development, New York, NY, USA

Background: Use of long-acting injectables (LAIs) has the potential to simplify tuberculosis (TB) preventive therapy (TPT) addressing issues such as pill burden and adherence. Bedaquiline (BDQ) is a key drug in new short-course regimens for treatment of rifampin-resistant TB and could provide a short-course "pan"-TPT option for drug-susceptible and rifampin-resistant infections. BDQ and other diarylquinolines (DARQ) have pharmacological and pharmacokinetic (PK) properties amenable to LAI formulation. The aim of this work was to preclinically characterize a new more potent DARQ LAI as a single phase spray dried nanosuspension LAI formulations for use in TPT.

Methods: Single intramuscular dose PK profiles for 3 DARQ LAI formulations (formulations A-C) were characterized in mice. Based on mouse PK, formulation B was tested for bac tericidal activity in a validated BALB/c mouse model of TPT at single intramuscular (IM) doses of 62.5, 125 and 250 mg/kg, with the goal of maintaining ≥36 ng/mL plasma target for 4-8 weeks. Formulations A and C were tested only at 125 mg/kg. Negative controls were untreated. Positive controls received daily (5 days/week) oral isoniazid-rifapentine (1HP), oral BDQ or oral DARQ for 4 weeks. Lung bacterial colony-forming units (CFU) counts and plasma DARQ exposures were measured 4-12 weeks after the start of treatment. Group mean CFU counts were analyzed using 1-way ANOVA and plasma exposure with non-compartmental PK analysis.

Results: All DARQ LAI regimens had bac tericidal activity over the first 4 weeks of treatment that was superior to 1HP (p<0.0001) and oral BDQ (p<0.0001 except p=0.05 for 62.5 mg/kg) and similar in magnitude to the same total DARQ dose given orally over 4 weeks (Fig). Activity was dose-dependent for formulation B and similar for the 3 formulations at 125 mg/kg. Median DARQ Cmax was <1 μg/mL. Median AUC0-4w values were 137, 186 and 349 μg·h/mL and plasma concentrations were ≥36 ng/mL for 4, 6 and >6 weeks after the 62.5, 125 and 250 mg/kg doses of formulation B, respectively. Lung CFU and PK data at 8 and 12 weeks post-dose are pending.

Conclusion: These data provide proof-of-concept for a highly efficacious pan-TPT regimen comprised of a single IM dose of an LAI DARQ formulation. Further preclinical development including studies to define the PK target, human dose estimates and GLP toxicology evaluation is highly warranted.

Monocyte Activation in Persons With HIV and Latent TB Co-Infection in the ACTG A5279/BRIEF TB Trial

Moises A. Huaman1, Manuel G. Feria Garzen2, Ashley McKinnan1, Xinyu Du3, Khunchari Supparatpinyoo1, Claire A. Chougnet4, Michelle A. Kendall5, Frederick R. Sawel6, Kristine M. Erlandson7, Netanya S. Utau8, Michael M. Lederman9, Susan Swindell9, Amita Gupta10, Richard E. Chaisson11, Carl J. Fichtenbaum12
1University of Cincinnati, Cincinnati, OH, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3Chung-Ma University, Chung-Mai, Thailand, 4Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA, 5Kenyatta Medical Research Institute, Nairobi, Kenya, 6University of Colorado, Aurora, CO, USA, 7University of Texas Southwestern, Dallas, TX, USA, 8Case Western Reserve University, Cleveland, OH, USA, 9University of Nebraska Medical Center, Omaha, NE, USA, 10The Johns Hopkins University, Baltimore, MD, USA

Background: Latent Tuberculosis infection (LTBI) has been linked to increased immune activation and cardiovascular risk. We investigated the activation profile of monocytes from persons with HIV (PWH) who participated in the ACTG A5279 trial, a phase III trial of 4 weeks of daily rifapentine (RFP)/isoniazid (INH) versus 9 months of daily INH as a TB prevention therapy (TPT).

Methods: We analyzed available cryopreserved PBMCs obtained at baseline (pre-TPT) and at week 48 (post-TPT) from A5279 participants on antiretroviral therapy, with HIV viral load ≤200 copies/mL, and a tuberculosis skin test (TST) or interferon-γ release assay (IGRA) result at entry. Unstimulated, thawed PBMCs were stained with markers of monocyte subsets (CD14, CD16), activation (HLA-DR, CD64, CD80, CD163), chemokinesis (CX3CR1,CCR2), and lipid uptake (CD36,CD163). In vitro stimulation with lipopolysaccharide (LPS) was performed to evaluate monocyte markers, and IL-6 and TNF-α expression. Samples were examined with multiparameter flow cytometry. Primary comparisons were between TST/IGRA-positive (evidence of LTBI) and TST/IGRA-negative groups. Linear regression of logtransformed markers adjusted comparisons for age, sex at birth, country, and CD4 count.

Results: 58 participants from 4 countries were included. Median age was 38 years (IQR, 34 – 47), 33 (57%) men and 25 (43%) women. At baseline, compared to TST/IGRA-negative participants (n=27), those with LTBI (n=31) exhibited higher percentage and/or median fluorescence intensity (MFI) of CD64 and CCR2 on their total and classical monocytes. At week 48, compared to TST/IGRA-negative group, participants with LTBI had higher percentage and/or MFI of CD64 on total and all monocyte subsets, as well as differential expression of CD80, CD163, and CX3CR1 across some monocyte subsets. Upon LPS stimulation, there were no differences in IL-6 or TNF-α production by TST/IGRA status; however, LTBI was associated with higher MFI of CD64 and CCR2, and lower MFI of CX3CR1 on total monocytes and subsets. In adjusted analyses, LTBI was consistently associated with increased MFI of CD64 (unadjusted) and CCR2 (post-LPS) at baseline and week 48 across monocyte subsets (Table).

Conclusion: Compared to PWH with negative TST/IGRA, PWH with evidence of LTBI exhibited monocyte alterations indicative of persistent activation and tissue migration at baseline and week 48. Longitudinal changes will be evaluated in future studies.

Single Cell Transcriptomics Reveals Depletion of TB-Specific Th1 and Th17 Cells After HIV Infection

Rachel A. Pearson1, Krista N. Krish1, Wendy Whatney1, Walter Jaoko2, Kishor Manda1iya1, Julie Overbaugh1, Susan M. Graham1, R. Scott McClelland1, Sakeenah Hicks1, Jeffrey Maurer3, Christopher D. Schairer1, Cheryl L. Day4
1Emory University, Atlanta, GA, USA, 2University of Nairobi, Nairobi, Kenya, 3PathCare, Mombasa, Kenya, 4Fred Hutchinson Cancer Center, Seattle, WA, USA, 5University of Washington, Seattle, WA, USA

Background: HIV substantially increases the risk of progression of Mycobacterium tuberculosis (Mtb) infection to active tuberculosis (TB) disease. This heightened risk of developing TB persists even in people with suppressed viral loads on long-term antiretroviral therapy (ART). The mechanisms underlying the loss of immune control of Mtb in people with HIV (PWH) remains unclear. We hypothesized that HIV dysregulates Mtb-specific CD4 T cells and that initiation of ART early following HIV infection will better preserve their functional capacity.

Methods: PBMCs were collected from a longitudinal cohort of women who engage in sex work in Mombasa, Kenya. PBMCs were evaluated from women within one year before and after HIV acquisition (n=5), and from women with HIV before and after ART initiation (n=8). PBMCs were incubated overnight with Mtb whole-cell lysate, followed by FACs sorting to purity CD40L+CD69+ Mtb-specific CD4 T cells for single-cell RNA-sequencing (scRNA-seq) using the 10X Genomics platform.

Results: Unsupervised clustering of scRNA-seq data revealed that distinct populations of Mtb-specific Th1 and Th17 cells are depleted after HIV infection. Differential gene expression analysis indicated decreased expression of genes encoding multiple effector molecules, including IFNG, IL22, TNF, IL17F, GZMB, GZMA, and GZFH, in Mtb-specific CD4 T cells in the first year after HIV infection, compared with Mtb-specific CD4 T cells from the same individuals before HIV infection. Moreover, PWH who initiated ART within 6 months of HIV infection exhibited increased proportions of Th1 and Th17 cells following ART compared with before ART, whereas recovery of Mtb-specific Th1 and Th17 cells was not observed in PWH who initiated ART >1 year after HIV acquisition. Additionally, multiple cytokine genes, including IFNG, IL2, IL22, and TNF, were downregulated in Mtb-specific CD4 T cells from PWH who started ART later (>1 year), compared with those who initiated ART earlier (<6 months) after HIV acquisition.

Conclusion: These novel single-cell transcriptomic studies indicate that HIV acquisition is associated with dysregulation of the Mtb-specific CD4 T cell transcriptome and depletion of crucial Th1 and Th17 cell populations within the first year after HIV infection, which may contribute to compromised Mtb control in PWH. Moreover, these results suggest that ART initiation within 6 months of HIV acquisition may be beneficial in preserving Mtb-specific Th1 and Th17 cells in PWH.
884 Persistence of PD-1+ Mtb-Specific CD4+ T Cells Is Associated With CAD During and After TB Treatment
Manuel G. Feria-Garzon1, Eduardo Ticona2, Cecilia Chang3, Wendy Guevara4, Anissa Moussa5, Alberto La Rosa1, Javier R. Lama4, Claire A. Chougnet2, Maises A. Huaman3, and Moussa1

1University of Cincinnati, OH, USA, 2Hospital Nacional Dos de Mayo, Lima, Peru, 3Association Civil Impacta Salud y Educacion, Lima, Peru, 4Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Background: Individuals who recovered from tuberculosis disease (post-TB) remain at increased risk of cardiovascular events and mortality. However, the underlying mechanisms of cardiovascular disease in post-TB remain unknown. Here, we aimed to characterize the immune profile of Mtb-specific CD4+ T cells in post-TB and explore their relationship with coronary artery disease (CAD).

Methods: We conducted a cross-sectional study of individuals 40 to 70 years of age with latent TB infection (LTBI), TB patients on isoniazid/rifampin consolidation treatment (TB-on-treatment); and clinically-cured individuals within one year of TB treatment completion (post-TB) between March 2018 and October 2019. Participants completed a coronary tomography angiography to assess CAD and provided blood for immune profiling of Mtb-specific CD4+ T cells using flow cytometry. Mtb-specific T cells were defined based on IFN-γ, IL-2, or TNF-α intracellular cytokine production upon in vitro PBMC stimulation with CFP-10/ESAT-6 or Mtb-whole-cell-lysate.

Results: 41 LTBI, 24 TB-on-treatment, and 11 post-TB participants without HIV were included in this analysis. After PBMC stimulation with Mtb-lysate, TB-on-treatment and post-TB patients exhibited higher percentage of Mtb-specific CD4+ T cells compared to LTBI (1.35 vs. 1.55 vs. 0.69; p=0.006). The percentage and MFI of activation markers HLA-DR, CD38, and delta HLA-DR (CD4minusMtb) were higher on Mtb-specific CD4+ T cells from TB-on-treatment and post-TB patients upon CFP-10/ESAT-6 and Mtb-lysate stimulation. Compared to LTBI, percentage of KI-67 and PD1 expression on Mtb-specific CD4+ T cells were increased in TB-on-treatment and post-TB upon Mtb-lysate stimulation (Figure 1). Polymorphism analyses revealed that TB-on-treatment participants exhibited a higher percentage of Mtb-specific CD4+ T cells positive to ≥2 cytokines upon Mtb-lysate stimulation, compared LTBI and post-TB participants. TB-on-treatment and post-TB individuals with CAD had higher MFI of PD1 on Mtb-specific CD4+ T cells, compared to those without CAD (181.5 vs. 123; p=0.022). Similarly, MFI of PD1 on Mtb-specific CD4+ T cells from TB-on-treatment and post-TB individuals positively correlated with CAD-RADS score (Spearman r=0.05; p=0.017).

Conclusion: Despite clinical TB cure, individuals post-TB exhibited signs of persistent immune activation and exhaustion of their Mtb-specific CD4+ T cell population. PD1 expression on Mtb-specific CD4+ T cells was associated with CAD during TB treatment and post-TB.

885 Suppression of Immunoglobulin Production and Mortality in HIV-Associated Cryptococcal Meningitis
Ronan Doyle1, J. Kathryn Boyle1, David S. Lawrence1, Kwana Lechiile2, Thiboe B. Leeme2, Nabila Youssouf3, Thomas S. Harrison4, Cecilia Ranyama5, Mosespe Mosepele6, Henry C. Mwandumba1, Chiratidzo Ndlovu7, James S. Harrison8, Joseph N. Jarvis1, for the AMBITION Study Group

1London School of Hygiene & Tropical Medicine, London, United Kingdom, 2Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 3London School of Hygiene & Tropical Medicine, Blantyre, Malawi, 4St. George’s University of London, London, United Kingdom, 5University of North Carolina Project–Malawi, Lilongwe, Malawi, 6University of Botswana, Gaborone, Botswana, 7Malawi–Liverpool Wellcome Trust Clinical Research Programme, Blantyre, Malawi, 8University of Zimbabwe, Harare, Zimbabwe, 9University of Birmingham, Birmingham, United Kingdom

Background: Cryptococcal meningitis (CM) primarily affects those with advanced HIV disease. Regardless of antiretroviral therapy status, roughly 15-20% of those diagnosed with CM die within 2 weeks even with the best available antifungal treatment. Despite advances in the management of CM with combination antifungals, there is still a poor understanding of the possible compromised immune responses contributing to this high mortality. There have been very few studies in humans, and these have tended to focus on a handful of biomarkers. In this study, for the first time, we use whole transcriptome RNA sequencing to identify the gene expression signature in blood and CSF from those who died from CM compared to those who survived.

Methods: We performed bulk RNA-sequencing on whole blood and CSF collected from the first 200 consecutively recruited participants with HIV and CM into the AMBITION-cm trial in Botswana, Zimbabwe and Malawi. After sequencing we analysed differential gene expression and Gene Ontology pathway analysis from 99 CSF and 162 blood samples comparing those who died within 2 weeks (CSF; n = 10, Blood; n = 18) to those that survived at 2 weeks (CSF; n = 89, Blood; n = 144).

Results: We identified a robust transcriptional signature in the CSF where 1010 genes were significantly differentially expressed when comparing survivors to the deceased (Fig 1a). The majority of these differentially expressed genes (DEGs) were downregulated in participants who died within 2 weeks. Gene ontology analysis of these downregulated genes indicated that it was suppression of pathways associated with immunoglobulin production as well as complement and B cell activation that were all significantly associated with mortality (Fig 1b). From these pathways we identified 21 DEGs responsible for immunoglobulin production and structure expressed in participants that survived and absent in those that died. Analysis of whole blood did not reveal any significant transcriptional responses.

Conclusion: This study is the first of its kind to identify a unique CSF transcriptional signature that differentiates CM survivors from deceased. This was unique to CSF and was not seen in blood. We found an impaired adaptive immune response and diminished B cell response that is common in advanced HIV disease and that may lead to an inability to clear the fungus. This work can now be applied to develop better prognostic tests and improve targeted treatments for severe CM and other neurological infections.
Recent ART Initiation and Mortality Risk in HIV-Associated Cryptococcal Meningitis

Melanie Moyé, Newton Kalata, James Jafali, S. Lawrence, Sile Molloy, Henry C. Mwandumba, Johnstone J. Kumwenda, and Thomas S. Harrison

Background: Over half of patients diagnosed with cryptococcal meningitis are ART-experienced. The impact of recent ART initiation on outcomes in PLHIV who present with cryptococcal meningitis, and how to optimally manage ART, are unknown. We hypothesised that patients presenting with cryptococcal meningitis following very recent ART initiation (within 14 days) were at higher risk of mortality than ART-naïve individuals or those on ART for longer periods, and that ART interruption reduces this excess mortality.

Methods: We analysed data from the ACTA and AMBITION trials to assess whether patients diagnosed with cryptococcal meningitis within 14 days of ART initiation were at higher risk of mortality, and to evaluate the impact of ART interruption at clinical decision on mortality. ART interruption was only performed at clinicians’ discretion in the AMBITION trial. We used a generalized linear model to assess the association between ART status and duration of ART. A mixed-effects model was used to assess differences in mortality rates between groups.

Results: 1484 individuals were included, 707 (48%) not on ART and 777 on ART. ART timing data were available for 649 ART-experienced participants; 18% (120/649) initiated ART 14 days or less prior to diagnosis of CM. Patients who had initiated ART within 14 days had higher CD4+ counts, and higher fungal burden than those on ART for over 14 days. 2-week mortality was 21.7% (95% CI 14.3-29.0%) in those on ART for 14 days or less, 13.8% (95% CI 7.9-19.8%) on ART for 15-60 days, and 7.6% (95% CI 2.2-13.0%) on ART for 61 days-6 months, increasing to 16% (95% CI 12.2-20.4%) in those on ART for more than 6 months (compared to 16% in those not on ART). Similar mortality trends were seen at 10 weeks. There was a significant interaction between the clinical decision to interrupt ART at enrolment and ART timing on mortality risk (p = 0.01). Among individuals on ART for less than 14 days in the AMBITION trial, 2-week mortality was 35% (8/23) with continued ART versus 14% (7/42) in those who discontinued ART (p = 0.05).

Conclusion: Our findings show an increased risk of early mortality in cryptococcal meningitis patients who started ART in the past 2 weeks in keeping with prior preliminary data. ART interruption at CM diagnosis in recent ART initiators does not lead to increased mortality and may be associated with improved outcomes. Intervention studies are needed to definitively determine whether ART interruption in patients presenting with cryptococcal meningitis has recently started ART is beneficial.

886 CSF HIV Viral Escape Is Associated With Improved Survival in Adults With HIV-Associated Meningitis

Jayne P. Ellis, Biyue Dai, Laura Nasangi, Glia Hale, Emmanuel Mandé, Jane Gakuru, Enock Kagimu, Timothy Mugabi, Derrick Kasozi, Sara Kimuda, Asmus Tukundane, David B. Meya, David B. Boulware, Fiona Cresswell, Thomas S. Harrison, Laura Nsangi, Suzan Namombwe, Jane Gakuru, and Thomas S. Harrison

Background: The phenomenon of CSF HIV viral escape is likely a by-stander phenomenon that remains the most common causes of HIV-associated meningitis, accounting for >20% of AIDS-related deaths globally. Acute mortality associated with these infectious meningitides remains devastatingly high (25-50%), even in the context of clinical trials. We hypothesised that cerebrospinal fluid (CSF) HIV viral escape may contribute to central nervous system (CNS) damage and be associated with increased mortality.

Methods: We conducted a cohort study of HIV-positive Ugandan adults with suspected meningitis in Kampala, Uganda. We performed baseline paired plasma/CSF HIV viral load (VL) testing (Xpert; Cepheid, Sunnyvale, CA, USA). We determined the prevalence of CSF HIV viral escape was present in 30% (46/152) overall, and it was strongly associated (p<0.001) with higher CSF white cell count (102 vs. <5 cells/mcL); and shorter duration of ART (16 vs. 144 days). Amongst those on ART for >100 days, CSF HIV viral escape prevalence was 60%. CSF HIV viral escape was associated with improved 18-week survival with an unadjusted Hazard Ratio 0.41 (95%CI, 0.18 – 0.92, p=0.031). CSF viraemia alone was not associated with survival (unadjusted Hazard Ratio 1.04, 95%CI 0.52 – 2.09, p=0.9).

Conclusion: In our HIV-associated meningitis cohort, CSF HIV viral escape was common, especially amongst participants recently initiated on ART. As HIV viral replication suppresses more slowly in CSF than plasma, in such cases it is possible that with longer ART duration the CSF would also suppress. Given the observed associations with CSF pleocytosis and improved survival, in the context of HIV-associated meningitis, CSF HIV viral escape is likely a by-stander phenomenon and an indicator of effective host immune response with trafficking of monocytes and C4d positive cells.

Table 1: Baseline characteristics and survival outcomes of 152 adults with HIV-associated meningitis on ART initiation (with stratification by CSF HIV viral escape status)

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>CSF HIV viral escape (median [IQR])</th>
<th>No CSF HIV viral escape (median [IQR])</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART status</td>
<td>148</td>
<td>24 (13-32)</td>
<td>41 (34-60)</td>
<td>0.02</td>
</tr>
<tr>
<td>ART days</td>
<td>&lt;100 days</td>
<td>21 (7-57)</td>
<td>43 (20-85)</td>
<td>0.11</td>
</tr>
<tr>
<td>CSF viral load (copies/ml)</td>
<td>120</td>
<td>744 (14-3200)</td>
<td>320 (10-3200)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF CD4 cell count (cells/ml)</td>
<td>99</td>
<td>55 (44-102)</td>
<td>55 (33-860)</td>
<td>0.8</td>
</tr>
<tr>
<td>CSF white cell count (cells/ml)</td>
<td>120</td>
<td>1.4 (1.0-3.0)</td>
<td>1.0 (0.5-2.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log10(CSF viral load) (copies/ml)</td>
<td>122</td>
<td>4.7 (4.1-5.0)</td>
<td>4.0 (1.0-5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2-week mortality</td>
<td>122</td>
<td>31 (25-37)</td>
<td>31 (20-40)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

For categorical variables, the p-value is from Fisher’s exact test. For non-categorical variables, the p-value is from the Wilcoxon rank sum test.
**Quantitative Antifungal Activity of Daily Liposomal Amphotericin With SFC in Cryptococcal Meningitis**

David R. Boulware1, B'Nyue Dai1, Laura Nsangi2, Jane Gakuru2, Enock Kagimu3, Enos Kigozi2, Timothy Mugabi2, Derrick Kasozi2, Suzan Namombwe2, Sara Kimuda1, Jayne P. Ellis1, Caleb Skipper1, Ann Fieberg2, Conrad Musorza2, David B. Meya2

1 University of Minnesota, Minneapolis, MN, USA, 2 Infectious Diseases Institute, Kampala, Uganda, 3 Mahara University of Science and Technology, Mbarara, Uganda

**Background:** Daily liposomal amphotericin B (AMB) with flucytosine (SFC) is recommended as first-line therapy in US cryptococcal meningitis treatment guidelines. Liposomal AMB monotherapy at 3mg/kg/d in its FDA registration did not meet the prespecified non-inferiority criteria of <10% difference in 10-week survival or 10-week culture conversion rate compared to AMB deoxycholate. Daily liposomal AMB combination therapy with SFC, although recommended, has not been studied in a clinical trial. We sought to assess the quantitative antifungal activity of this first-line recommended regimen.

**Methods:** We enrolled Ugandans with HIV-related cryptococcal meningitis into prospective cohorts and clinical trials from 2018-2023. We assessed the early fungicidal activity (EFA) of the cerebrospinal fluid (CSF) Cryptococcus clearance rate between those receiving AMB deoxycholate 1mg/kg + SFC 100 mg/kg/d versus those receiving daily liposomal AMB 3 mg/kg/d + SFC 100 mg/kg/d. induction AMB + SFC was given for 7 days, followed by fluconazole 1200 mg/d. We calculated EFA by linear regression from longitudinal quantitative CSF fungal cultures collected over 2 weeks of therapy.

**Results:** Among 199 participants with longitudinal quantitative CSF culture data, 156 received AMB deoxycholate, and 43 received liposomal AMB. For AMB deoxycholate, the mean EFA was 0.403 (95%CI, 0.36-0.44) log CFU/mL/day. For daily liposomal AMB, the mean EFA was 0.493 (95%CI, 0.35-0.64) log CFU/mL/day. The absolute EFA difference was 0.090 (95%CI, -0.02 to 0.20) log CFU/mL/day favoring liposomal AMB. Grade ≥3 adverse events were less frequent among those receiving liposomal AMB.

**Conclusion:** Despite never being quantified in a randomized clinical trial, the combination of liposomal AMB with SFC had good antifungal activity in humans with cryptococcosis which did not statistically differ from that of AMB deoxycholate.

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**IgG Responses Are Associated With Severe Disease and Mortality in AIDS-Associated Talaromycosis**

Shanti Narayanasamy1, Matthew T. Burke1, Ngo Thi Hoa1, Thuy Le1, Thu T. Nguyen1, Vo Trieu Ly1

1 Duke Global Health Institute, Durham, NC, USA, 2 Duke University School of Medicine, Durham, NC, USA, 3 Oxford University Clinical Research Unit in Vietnam, Ho Chi Minh City, Vietnam, 4 Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

**Background:** There is dearth of data on antibody responses to AIDS-associated talaromycosis and how they impact disease phenotype and patient outcomes. Data from our talaromycosis Tracarosazone versus Amphotericin B for Penicilliosis (IVAP) trial (N=440) showed that patients presenting with an acute pulmonary syndrome had higher mortality. Here, we tested the hypothesis that acute pulmonary syndrome is associated with a serological pattern of acute infection, and an acute serological pattern is associated with severe disease and mortality.

**Methods:** Longitudinal IgG antibody responses to talaromycosis were measured in available plasma samples of IVAP patients using a direct anti-Mtp1 IgG enzyme immunoassay. Patients were classified into three IgG response types: Negative IgG, Increasing IgG, and Positive Unchanged IgG through analysis of the IgG trend over 24-weeks. Univariate and multivariate logistic regression were used to assess association of IgG type and disease severity (defined as a respiratory rate ≥22, dyspnea requiring oxygen, and/or respiratory failure diagnosis at presentation) and 24-week mortality.

**Results:** Plasma samples and complete data were available for 409 of 440 IVAP patients. Mean age was 34.7 years (SD:7.4). Mean CD4 count was 23.3 cells/ mm3 (SD:49.1). Serial samples for classification of IgG response into 3 types were available for 312 patients: 88 (28.2%) Negative IgG, 114 (36.6%) Increasing IgG, 110 (35.3%) Positive Unchanged IgG. In the multivariate models of the 312-subpopulation (N=312) adjusting for age, history of injection drug use, CD4 count, and antifungal treatment arm, Negative IgG was associated with higher risk of death (OR=3.20,95%CI:1.18-8.96, P=0.023) while Increasing IgG was protective of death (OR=0.145,95%CI:0.02-0.56, P=0.013). In the multivariate models of the full population (N=409) where IgG type could only be classified as Negative or Positive at baseline, Negative IgG was associated with increased disease severity (OR=1.62,95%CI:1.02-2.55, P=0.039) and had a non-statistically significant association with mortality. Disease severity was an independent predictor of death (OR=2.44,95%CI:1.38-4.34, P=0.002).

**Conclusion:** Although these results were unexpected, the findings that 28% of talaromycosis patients do not mount an IgG response, and these patients have more severe disease and higher mortality suggest a central role of humoral immune response in talaromycosis pathogenicity, which has so far been overlooked. These findings have important clinical implications.

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889 Triple Screening for Invasive Mycoses in Patients with Advanced HIV Disease in Vietnam

Vu Quoc Dat1, Dieu Q. Nguyen1, Hao T. Nguyen1, Vo Trien Ly1, Thach N. Pham1, Do Duy Cuong5, Nam X. Ha4, Tran Thi Hong Chau4, Phuong L. Trinh5, Kha H. Dang5, Phan Thi Hong Dau5, Trinh Thi Men5, Nguyen Thi Hoai Dung6, H. Rogier van Doorn1, Thuy Le6, for the Talaromycosis Study Group

1 Hanoi Medical University, Hanoi, Vietnam, 2 Oxford University Clinical Research Unit in Vietnam, Ho Chi Minh City, Vietnam, 3 Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, 4 National Hospital for Tropical Diseases, Hanoi, Vietnam, 5 Bach Mai Hospital, Hanoi, Vietnam, 6 Oxford University Clinical Research Unit in Vietnam, Ho Chi Minh, Vietnam, 7 Duke University School of Medicine, Durham, NC, USA

**Background:** Invasive mycoses are second to TB as a leading cause of death in advanced HIV diseases (AHD). The WHO recommends screening for TB and cryptococcosis in a package of care for AHD; however, invasive mycoses endemic in Asia Pacific - talaromycosis and histoplasmosis - are not included due to the lack of diagnostics and data on burden of diseases to inform policy. We present the results of a triple fungal screening program for in patients with AHD in Vietnam.

**Methods:** This is an ongoing multi-center prospective fungal screening cohort study of patients with AHD who presented for hospitalisation (cohort 1, n=900) or for ART initiation in outpatient clinics (cohort 2, n=600) in three hospitals in Hanoi and Ho Chi Minh city, Vietnam. We enrolled patients aged ≥18 years, with CD4 count ≤100 cells/ mm3 or WHO stage 3 or 4, not on ART OR recent ART ≤3 months OR suspected or confirmed treatment failure on ART ≥12 months. We excluded patients currently on effective systemic antifungal treatment. All patients received antigen screening for cryptocoecosis using the IMMY Cryptococcal antigen lateral flow assay (CrAg LFA) in blood, histoplasmosis using the IMMY Clars Histoplasma GM enzyme immunnoassay (Hag EIA) in urine, and talaromycosis using a novel in-house Mtp1 EIA (TmAg EIA) in blood and urine, alongside conventional diagnostics including BACTEC and MycoF/Lytic blood cultures. Patients were followed up monthly over 6 months (cohort 1) and 12 months (cohort 2) to assess outcomes.

**Results:** 1366 patients were enrolled, including 900 hospitalised patients (100% target for cohort 1) and 466 outpatients (77.7% target for cohort 2) between February 2021 and August 2023. In cohort 1, the median age was 36 years (IQR: 30–44), 746 (82.9%) were men. The positivity for fungal antigens was 18.6% (95% CI: 16.1-21.2%) for TmAg, 4.3% (95% CI: 3.1-5.8%) for CrAg, and 4.1% (95% CI: 3.0-5.6%) for HagAg. Among TmAg-positive patients, 6.6% (95%CI 3.5-11.1%) were HagAg-positive and 1.8% (95%CI 0.5-4.7%) were CrAg-positive. In cohort 2, the positivity for fungal antigens was 8.4% (95% CI: 6.1-11.1%) for TmAg, 3.9% (95% CI: 2.4-5.9%) for CrAg, and 2.1% (95% CI: 1.1-3.8%) for HagAg. The analysis of clinical outcome is ongoing.

**Conclusion:** Invasive mycoses were detected in 25% of hospitalised and 14% of outpatients with AHD in Vietnam. Talaromycosis is the leading mycosis and
891 Enhancing Advanced HIV Screening: Innovating With the "Hub and Spoke" Testing Model in Tanzania

Nelson M. Jonas1, Alexander Christopher1, Christopher2, Amos Scott1, Julius Zesthe1, John Roman1, Aaron Tesh1, Frederick Ndossi1, Christopher Jenyewe1, Josephat Franc1, Peter Mlacha1, Hosea William1, Andrea Mbunda1, Eva Matiko1, Redempta Mbatia1, redempta mbatia1, T. Tanzania Health Promotion Support, Dar es Salaam, United Republic of Tanzania, 2. Tanzania Health Promotion Support, Shinyanga, United Republic of Tanzania, 3. Tanzania Health Promotion Support, Kigoma, United Republic of Tanzania, 4. Tanzania Health Promotion Support, Pwani, United Republic of Tanzania, 5. Tanzania Health Promotion Support, Dar es Salaam, Ministry of Health, United Republic of Tanzania, 6. Tanzania Health Promotion Support, Kilimanjaro, United Republic of Tanzania, 7. US Centers for Disease Control and Prevention Tanzania, Dar es Salaam, United Republic of Tanzania

Background: Despite the global adoption of the World Health Organization’s ‘test and treat’ policy, proportion of people living with HIV (PLHIV) presenting with advanced HIV disease (AHD) remains high at around 30% in Tanzania. Due to gaps in identification, recipients of care (RoC) with AHD face an elevated risk of mortality primarily because of opportunistic infections (OIs) such as tuberculosis and cryptococcal meningitis (CM). We demonstrated the implementation of AHD screening through CD4 testing to detect and manage OIs, particularly cryptococcal infection.

Methods: In collaboration with the Kigoma, Pwani, and Shinyanga Regional and Council Health Management Teams, we revisited CD4 testing for all newly diagnosed PLHIV along with reflex cryptococcal antigen (CrAg) testing. We extracted data from the HIV Care and Treatment Clinic database, comparing the identification of AHD after the intervention through CD4 and CrAg testing. In 2019, we integrated CD4 testing within the established viral load sample transportation system using a ‘hub and spoke’ model and point of care (PoC). Healthcare providers received training on AHD screening and management, including CD4 testing for all newly enrolled to care and laboratory-reflex CrAg testing at the laboratory for all samples with CD4 counts of <200 cells/μL. We used the client’s recorded cards and care and treatment database as the main data source. The analysis focused only on newly enrolled RoC.

Results: Enhancing sample transportation led to 167 (68%) supported facilities without CD4 machines to access CD4 testing and cryptococcal screening through CrAg tests at the hub sites. The number and proportion of newly enrolled RoC tested for CD4 at 245 supported facilities rose from 1,931 (7%) in 2020 to 9,900 (95%) in 2023. Simultaneously, the proportion of samples with CD4 counts < 200 undergoing laboratory-reflex CrAg testing increased from 173 (36%) in 2020 to 1,474 (96%) in 2023, resulting in CrAg positive identification of 35 (20%) individuals in 2020, 89 (16%) in 2021, 102 (10%) in 2022, and 90 (6%) as of June 2023. CM cases identified increased from 5 in 2020 to 39 in 2023.

Conclusion: The CD4 testing for newly diagnosed HIV-positive clients has substantially increased the identification of individuals with asymptomatic cryptococcal infection. The successful implementation of early AHD screening among newly diagnosed PLHIV in resource-constrained settings is feasible through sample transportation integration and expanding point of care CD4 testing.
obtaining fungal loads requires serial CrAg LFA dilutions or quantitative fungal cultures (QFC), both are time and resource consuming, need expertise, and are difficult to implement in resource limited settings (RLS). The IMMY CrAgSQ is an improvement on the widely used IMMY CrAg LFA with comparable diagnostic performance, yielding a semi-quantitative (SQ) score. Previous studies involved fewer HIV-CM patients and did not definitively evaluate the utility of CrAgSQ in predicting mortality. We conducted a study on stored plasma and CSF samples from the AMBITION trial to evaluate whether CrAgSQ can predict outcomes.

Methods: 810 patients with HIV-CM were enrolled onto the AMBITION Phase 3 randomised clinical trial evaluating CM therapy in Botswana, Zimbabwe, Malawi, Uganda and South Africa. Stored plasma and CSF samples collected at disease diagnosis before treatment were tested using CrAgSQ, yielding a score of 1-5. Clinical outcomes and laboratory data were documented during 10 weeks of follow-up. Associations between CrAg SQ scores in plasma and CSF and 2- and 10-week mortality were examined.

Results: 756 CSF and 745 plasma collected from 796 AMBITION Trial patients were assessed; 61% of the patients were male, median age was 37 (IQR 32-43 years), and median CD4 count was 27 (IQR 10-58 cells/µL). Overall mortality was 13% and 23% by 2 and 10 weeks. In plasma, 0.13% (1/745) were negative, 4.03% (30/745) had an SQ score of 1, 3.49% (26/745) 2, 2.96% (22/745) 3, and 8.72% (65/745) 4. In CSF, 1.32% (10/756) were negative, 7.01% (53/756) had an SQ score of 1, 5.66% (43/756) 2, 6.37% (48/756) 3, and 22.22% (168/756) 4. CrAg SQ scores in plasma (Figure) and CSF positively correlated with QFCs. Increasing CrAg SQ scores in plasma were strongly associated with increasing mortality risk at 2- and 10-weeks. Mortality was 0% (0/22) in those with plasma SQ score of 1, 4.4% (1/23) 2, 13.9% (75/540) 3, and 34.55% (19/55) 4 (p for trend <0.001).

Conclusion: Plasma CrAgSQ is a promising prognostic tool for 2-week mortality in patients with HIV-CM, making it a viable option for RLS. Further research is warranted to establish how it could be used to stratify management in individuals presenting with HIV-CM.

Histoplasmosis in Advanced HIV Disease: A Multi-Center Prospective Diagnostic Validation Study

Hao T. Nguyen, Dieu O. Nguyen, Vo Trieu Ly, Khanh D. Dang, Phuong L. Trinh, Thach N. Pham, Nguyen Thanh Dung, Nguyen Thi Lan, Do Thi Le Na, Nguyen Thanh Vinh, Nguyen Phu Huong Lan, Vu Quoc Dat, H. Rogier van Doom, Thuy Le, for the Talaromyces Study Group

Background: Histoplasmosis burden in advanced HIV disease (AHD) in Southeast Asia is unknown due to the lack of diagnostics. We report results of an ongoing multi-center prospective histoplasmosis diagnostic validation study in patients with AHD in Vietnam.

Methods: We recruited hospitalized HIV-infected adults, CD4 count < 100 cells/µL or WHO stage 3 or 4 disease, ART-naive or on ART for ≤ 3 months or > 12 months from 3 hospitals in Vietnam. All patients were screened for Histoplasma antigen using the IMMY Clarus Histoplasma GM enzyme immunoassay (HAg EIA) in urine and Myco/F Lytic blood culture (over 6 weeks), alongside conventional microscopy and cultures of other specimens as clinically indicated. Proven histoplasmosis was defined as culture-positive disease. Probable histoplasmosis was defined as a compatible clinical syndrome and resolution of HAg levels on antifungal therapy. Patients were followed up monthly for over 6 months.

Results: 900 patients were recruited between 02/2021 and 08/2023. Overall prevalence of HAg positivity was 37/900 (4.1%) and was higher in southern vs. northern Vietnam, 30/581 (5.2%) vs. 7/319 (2.2%), P= 0.035, Fisher Exact.

Among the 37 HAg-positive patients, 15 (40.5%) had proven histoplasmosis; 13 (35.1%) had probable histoplasmosis; and 9 (24.3%) did not develop disease over 6 months. The median HAg EIA unit of proven and probable histoplasmosis patients was 35.84 (IQR 4.31 to 40.96) compared to -0.04 (IQR -0.08 to 0.03) of non-histoplasmosis patients, P<0.001, Mann-Whitney. At a cut-off EIA unit of 1.2, HAg EIA had 100% sensitivity (95% CI: 87.7% - 100%), 99% specificity (95% CI: 98.1% - 99.5%), 75.7% positive predictive value (95% CI 61.9% - 85.6%), 100% negative predictive value (95% CI 99.6% - 100%), and an accuracy of 99.8% (95% CI: 99.7% - 100%). Among 28 histoplasmosis patients, the median CD4 count was 17 (IQR, 7 - 31) cells/µL. H. capsulatum was isolated from blood in 12 (42.9%), bone marrow in 11 (35.3%), skin lesions in 1 (3.6%) patients. Median time to culture positivity in bone marrow and blood was: 10.5 (IQR – 7.15)
days and 17 (IQR, 11.5 – 19) days, P=0.166, Wilcoxon. 7/28 (25%) patients died by month 6 despite antifungal therapy.

**Conclusion:** Our study demonstrates an excellent diagnostic performance of the HAg EIA and unveils a substantial burden of histoplasmosis in AHD in Vietnam. The mortality remains high (25%) despite antifungal therapy, highlighting the need to screen for Histoplasma antigen, preferably before patients become symptomatic.

### Ultra Xpert in Blood and Urine and Myco/F Lytic Blood Culture in Patients With Advanced HIV Disease

Dieu O. Nguyen, Hao T. Nguyen, Thu D. Do, Quan L. Nguyen, Vu Quoc Dat, Phuong L. Trinh, Khanh H. Dung, Thach N. Pham, Nguyen Thanh Dung, Nguyen Phu Huong Lan, Vo Trieu Ly, Nguyen Thi Thuong Thuong, H. Rogier van Doorn, Thuy Le, for the Talaromycosis Study Group

**Background:** Tropical Diseases, Ho Chi Minh City, Vietnam, 1Oxford University Clinical Research Unit in Vietnam, Ho Chi Minh City, Vietnam, 2Hanoi Medical University, Hanoi, Vietnam, 3National Hospital for Tropical Diseases, Hanoi, Vietnam, 4University for Tropical Diseases, Ho Chi Minh City, Vietnam, 5Duke University School of Medicine, Durham, NC, USA

**Methods:** This sub-study was part of a triple fungal screening program in AHD (N=900 patients). Eligible patients included all hospitalized adults aged ≥18 with CD4 count ≤ 100 cells/µL or WHO stage 3 or 4 disease, not on ART or were on ART for ≥ 3 months or >12 months from two major hospitals for tropical diseases in Hanoi and Ho Chi Minh City. Intensified screening approach included blood UXpert and MFL blood culture in all patients. Urine UXpert was an add-on for the last 174 patients. Conventional approach included acid-fast stain, UXpert and MGIT culture of sputum and other specimens as clinically indicated. Patients were followed up monthly over 6 months. Diagnostic yield was compared between two approaches.

**Results:** The 900 patients were recruited between February 2021 and July 2023. 82.9% were men. Median age was 36 (IQR: 29-44) years. A total of 301 (33.4%) patients had microbiological-confirmed mycobacterial infections: 243 (80.7%) had TB; 30 (10.0%) had non-TB mycobacteria (NTM) - 24/30 (80.7%) had M. tuberculosis, 27/30 (90.9%) had M. avium complex. The number of cases diagnosed by conventional, intensified, and MGIT culture of sputum and other specimens as clinically indicated. Patients were followed up monthly over 6 months. Diagnostic yield was compared between two approaches.

**Conclusion:** The intensified screening approach using UXpert in blood and urine and MFL blood culture increased the number of mycobacterial diagnoses by 54% compared to conventional method alone in hospitalized patients with AHD. Implementation of UXpert in blood and urine in routine care is feasible, reduces time to diagnosis and treatment, and has the potential to reduce HIV mortality.
pregnant women had objective levels of PrEP use (any TFV-DP in DBS). Women with TFV-DP present were older, >20 weeks GA (vs <20 weeks) at PrEP start, had a partner living with HIV (or unknown serostatus), had higher sex frequency in past month (>5 times vs <5 times or no sex) (p<0.05). Women with missing PrEP exposures had the highest rates of adverse pregnancy outcomes during the study period at 32.6% (18.4% pregnancy loss), 10.4% preterm delivery, 8.7% infant born SGA). In women with TFV-DP in their DBS (n=181), adverse outcomes were significantly lower at overall composite outcome at 17%, including 3.9% had pregnancy loss, 6.6% had preterm delivery, 6.6% had an infant born SGA (Table 1). Correlation between self-reported PrEP use and TFV-DP was −0.07 in pregnancy.

Conclusion: The proportion of “PrEP-exposed” pregnant women with adverse pregnancy outcomes differed significantly based on how PrEP exposure was defined in the study, highlighting the importance of objective monitoring in pregnancy safety studies as self-reported PrEP use did not correlate with objective levels. Pregnant women who discontinued PrEP had poorest pregnancy outcomes and may require additional interventions for PrEP and pregnancy health. Rapid, cost-effective methods for measuring objective PrEP use are urgently needed.

**Pharmacokinetic Study Comparing TAF and TDF as PrEP in Pregnant and Postpartum Women in South Africa**

Dvora L. Joseph Davey 1, Sumaya Dadan 2, Saye Khoo 2, Lulube Wiesner 2, Landon Myer 2, Peter L. Andersen 2, Catherine Orrell 2

1University of California Los Angeles, Los Angeles, CA, USA, 2University of Cape Town, Cape Town, South Africa, 3University of Liverpool, Liverpool, United Kingdom, 4University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 5Desmond Tutu HIV Foundation, Cape Town, South Africa

**Background:** ARVs for PrEP are effective at preventing HIV. However, among pregnant women receiving tenofovir disoproxil fumarate and emtricitabine (TDF/FTC), lower tenofovir (TFV) and intracellular tenofovir-diphosphate (TFV-DP) concentrations in red blood cells have been found compared to postpartum periods. No studies have been conducted to establish concentrations of TFV-DP after administration of tenofovir alafenamide (TAF) in pregnancy and postpartum individuals.

**Methods:** Between June 2022 and March 2023, we recruited eligible and consenting pregnant women from a cohort study in Cape Town, South Africa. We randomized participants in the 2nd trimester 1:1 to daily dosing of tenofovir alafenamide- emtricitabine (TAF/FTC) or TDF/FTC. Participants had 8 weekly study visits and bloods drawn (plasma, dried blood spots [DBS]) during pregnancy and again postpartum. Adherence was monitored using video directly observed therapy. We compared TFV-DP concentrations between TAF and TDF for pregnancy vs. postpartum in both DBS and PBMCs including geometric mean ratios (GMR) for low concentrations and 95% CIs (STATA v18).

**Results:** We evaluated 37 paired pregnancy and postpartum individuals; 19 in the TAF arm and 18 in the TDF arm. Two participants did not adhere to the protocol and were excluded. Median age was 28.2 years (IQR: 23-32); median gestation age at PrEP start was 24 weeks (IQR: 21-24 weeks). In TAF, median concentrations of TFV-DP in DBS were 51% higher in postpartum and 1% higher in PBMCs when compared to pregnancy. In TDF, median concentrations of TFV-DP in DBS were 12% higher in postpartum and 35% higher in PBMCs samples vs. pregnancy (Table 1). GMR for postpartum vs. pregnancy for TFV-DP was 1.68 (1.61-1.76) for TAF DBS, and 1.01 (0.94-1.07) for TAF PBMCs. GMR for TFV-DP in TDF DBS was 1.62 (1.53-1.71) for DBS and 1.39 (1.34-1.45) in PBMCs. In TAF samples, median FTC-TF was 9.42 (IQR:6.47-14.0) in pregnancy and 10.45 (6.64-14.45) in postpartum (GMR=1.15;1.11-1.99). Comparing TAF vs. TDF PBMCs, GMR was 30.1 (25.0-36.2), similar in pregnant and postpartum samples. No safety related adverse events were found.

**Conclusion:** Our findings demonstrate high concentrations of TFV-DP in PBMC in pregnancy and postpartum among women receiving TAF indicating PrEP efficacy is retained. DBS adherence benchmarks for daily dosing were established for TAF in pregnant and postpartum in African women.

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901 Video Observed Therapy for Preexposure Prophylaxis Use in South African Pregnant/Postpartum Women

Sumaya Dadan1, Catherine Orrell1, Peter L. Anderson2, Linda-Gail Bekker1, Rufaro Mvududu3, Moira Dyer2, Desmonde Twab for Paediatrics, South Africa, and Postpartum Women

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Background: Daily use of oral PrEP is essential for effectiveness. Video observed therapy (VOT) is an adherence technique involving daily observation of participant dosing via digital means. VOT is an alternative to directly observed therapy and may be useful for resource-scarce settings and pharmacokinetic (PK) studies. This study aims to evaluate the feasibility and acceptability of VOT in a PK study (PrEP PK) of oral PrEP in pregnant and postpartum South African women.

Methods: We analyzed data from adult pregnant and postpartum women collected over a year in the PrEP PK study. Each participant was observed for 16 weeks: 8 weeks in pregnancy, 8 weeks postpartum. Feasibility was defined as participant adherence to VOTs, reported as fraction of expected doses observed (FEDO). A median FEDO of ≥85% indicated VOT feasibility while <85% indicated lack of feasibility. Logistic regression analysis was used to determine the impact of baseline characteristics on FEDO score. Linear mixed effect models (LME) were used to examine the effect of observation time and pregnancy state on FEDO score. Acceptability was assessed through semi-structured questionnaires and interviews.

Results: Among n=53 women in the study, 39 (73.6%) completed all 16 weeks of observation, median(IQR) observation time: 15(14-16) weeks. Median age was 28(6). Of the 4571 videos expected 4112(90.0%) were received. Reasons for missed videos are in Table 1. Median(IQR) FEDO exceeded the feasibility threshold and was 91.4(82.9-97.1%). There was no significant impact of baseline characteristics on FEDO score. Median FEDO increased over weeks of observation: FEDO at week 1(81%); week 7(92%) and week 15(97%). On LME modelling, every additional week of observation time was associated with a 0.3%(95% CI: 0.20%-0.89%) increase in median FEDO score (p value= 0.002). During pregnancy, 94% of expected videos were received; 98% were received postpartum. There was no significant association between pregnancy state and FEDO score in LME modelling (p value= 0.523). When participants were asked about their experiences with VOT, 100% of respondents said that they would do VOTs again and 98% felt that it helped them adhere to PrEP.

Conclusion: VOT is feasible and acceptable in pregnant and postpartum women for monitoring and supporting daily PrEP adherence. A high proportion of women successfully completed the observation period. VOT was also a useful technique for assuring daily dosing; and can be applied to future PK studies that require strict adherence.

902 PrEP Discontinuation and High HIV Incidence in South African Pregnant and Postpartum Women

Rufaro Mwududzi, Kaisha Sheema1, Aurelie Nelson1, Linda-Gail Bekker2, Thomas Coates1, Landon Myer3, Dvora L. Joseph Davey1

University of Cape Town, Cape Town, South Africa, University of California Los Angeles, Los Angeles, CA, USA

Background: High HIV incidence is a major concern for pregnant and breastfeeding women (PBFW) in South Africa (SA) due to increased risk of HIV acquisition doubling during these periods. In 2019, the SA Department of Health guidelines were updated to include oral pre-exposure prophylaxis (PrEP) for PBFW, but availability of PrEP for PBFW is still limited in SA health facilities and PrEP discontinuation remains a challenge to effective use in this population.

Methods: In an antenatal clinic based in Cape Town, SA, the observational study recruited pregnant women without HIV between Aug 2019 – Oct 2021. The study provided HIV testing, counseling and offer of oral PrEP (TDF/FTC) to all pregnant women at their first visit, with follow-up through 12 months postpartum. At quarterly study visits study counselors conducted HIV testing and counselling to monitor serostatus of PBFW. In addition, HIV results, CD4 counts, and viral loads were reviewed for all study participants at the end of follow-up to confirm final serostatuses in electronic patient and laboratory files. We calculated HIV incidence rate using study follow-up person-time of Aug 2019 – Feb 2023. The numerator included seroconversions in study and lab results (incl. lost to follow-up [LTFU]). HIV incidence was stratified by pregnancy and postpartum time, using estimated delivery dates for those who were LT FU.

Results: Of the 1195 women enrolled, 1009 (84%) received a PrEP prescription and 82% confirmed PrEP initiation through self-report. After 12 months follow-up, 283 (26%) women continued PrEP use. Overall, 16 women (0.5%) seroconverted over 1684.8 woman-years of follow-up with an HIV incidence rate of 0.95/100 woman-years (95% confidence interval (CI) 0.58–1.55). Of 16 seroconversions, 1 woman (6%) reported taking PrEP in the past 3 months, 1 (6%) seroconverted during pregnancy, and HIV incidence was higher among women who had stopped or never started PrEP (1.89/100 woman-years vs 0.11/100 woman-years who continued PrEP). In the first 6 months postpartum, HIV incidence was 1.07/100 woman-years (95% CI 0.43–2.22) and 60% of the women seroconverted later in postpartum (>6 months) in 1.12/100 woman-years.

Conclusion: Despite the high PrEP initiation at enrolment, we observed a high HIV incidence rate in postpartum women who had started PrEP and discontinued, highlighting the importance of appropriate interventions targeting postdiscontinuation of PrEP through integration of PrEP delivery with services such as family planning and baby visits.

903 The Role of Maternal Broadly Neutralizing Antibody Activity in Perinatal Transmission of HIV-1

Kritika P. Kargiye1, Ashley Nelson1, Dieter Mielke1, Christian Binuya1, Ria Goswami1, John Isaac1, Elena Giorgi1, John Kapp,1,2, Jennifer Mace1,2,3, Megan Connors1,2,3, Carolyn Weinbaum1,2,3, Genevieve G. Fouda4, Feng Gao5, Manish Sagar4, Sharron Permall5, Sally Permall5,1,6,7,8

Fred Hutchinson Cancer Research Center, Seattle, WA, USA, Duke University, Durham, NC, USA, "Fred Hutchinson Cancer Research Center, Seattle, WA, USA, "Boston Medical Center, Boston, MA, USA

Background: Despite increased availability to antiretroviral therapy (ART), up to 5% of women living with HIV (WLH) still transmit the virus to their infants. While broadly neutralizing antibodies (bNAbs) are the immunologic goal of HIV-1 vaccine candidates, we have demonstrated escape of infant HIV variants in the presence of bNAbs targeting a single, dominant epitope in postnatal transmitters. We hypothesize that WLH with bNAbs responses against single epitopes of the HIV envelope are at higher risk of perinatal transmission due to viral escape and this impacts the development of a bNAb response in infants.

Methods: Plasma was acquired around delivery from 15 perinatal transmitters and 47 non-transmitters with HIV from the US-based, pre-ART era Mother-Infant Cohort Study (MICS), matched 1:3 on CD4+ T-cell counts, maternal age, and delivery type. Plasma from paired infants with HIV was acquired at 1-3 years to 5% of women living with HIV (WLH) still transmit the virus to their infants. While broadly neutralizing antibodies (bNAbs) are the immunologic goal of HIV-1 vaccine candidates, we have demonstrated escape of infant HIV variants in the presence of bNAbs targeting a single, dominant epitope in postnatal transmitters. We hypothesize that WLH with bNAbs responses against single epitopes of the HIV envelope are at higher risk of perinatal transmission due to viral escape and this impacts the development of a bNAb response in infants.

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904 Long-term Outcomes After Loss to Follow-Up From PMTCT Services for Women and Children in Kenya
John Humphrey1, Bett Kipchumba2, Edwin Sang1, Marsha Alera1, Beverly Musick1, Lindah Muli1, Justin Kipsang1, Julius Songok1, Constantin Yiannoutsos1, Kana Woolfolk-Kaloustian1
1Indiana University, Indianapolis, IN, USA; 2Moi Teaching and Referral Hospital, Eldoret, Kenya, 3Academic Model Providing Access to Healthcare, Eldoret, Kenya, 4Mois University, Eldoret, Kenya, 5Indiana University, Bloomington, IN, USA

Background: Many prevention of mother-to-child HIV transmission (PMTCT) studies assess outcomes within a year post-delivery and exclude patients who became lost to follow-up (LTFU) or transferred out, biasing outcomes toward those retained in care at the facility where they first enrolled in PMTCT services.

Methods: We recruited women living with HIV (WLH) ≥18 years that enrolled in antenatal clinic (ANC) at five public facilities in western Kenya. WLH retained in care (RW) were recruited during the 3rd trimester of pregnancy and followed from their children through 6 months post-delivery; WLH who became LTFU (LW, last visit >90 days) after ANC enrollment and before 6 months postpartum were recruited through community tracing. Re-contact at 3 years post-delivery was attempted for all WLH, using community tracing for WLH LTFU (>60 days since last missed scheduled visit before 36 months) and transferred. Primary outcomes of retention in care and child HIV-free survival were determined at 6 months and 3 years post-delivery.

Results: 333 WLH were recruited from 2018-2019. At 6 months postpartum, 222 WLH were classified as RW and 111 as LW (79 disenrolled from care, 32 silently transferred/retained elsewhere). More LW compared to RW were newly diagnosed with HIV at ANC enrollment (50% vs. 24%), not virally suppressed at study entry (40% vs. 6%), and miscarried (12% vs. 1%) (p<0.01 for all). HIV-free survival at 6 months was lower for children of LW vs. RW (88% vs. 99%, p<0.01). At 3 years, 230 WLH were retained at the study facility (81% of RW, 46% of LW). 30 officially transferred out (28 retained at a new facility, 2 unknown). 70 LTFU (8 silently transferred/retained elsewhere, 19 disenrolled, 43 unknown), and 3 deceased. Child HIV-free survival at 3 years was 82% (59% for children of LW, 92% for RW). 3.7% were living with HIV (11% LW, 0.4% RW), 3.7% were deceased (7% LW, 2% RW), and 11% had unknown HIV/vital status (23% LW, 5% RW).

Conclusion: HIV-free survival was lower for children of LW compared to RW at 6 months and 3 years post-delivery, emphasizing the need for interventions targeting early loss to follow-up from PMTCT services. Although most LW had re-engaged in care by 3 years, many remained LTFU and tracing-ascertained engagement in care was lower for WLH silently vs. officially transferred. Community tracing of patients who become LTFU can inform PMTCT outcome estimates and service delivery priorities for this population.

905 ART Adherence And Elevated Viral Load in Pregnant & Postpartum Women Initiating DTG Versus EFV
Thokozile R. Malaba1, Catherine Oreilli1, Laura Else1, Dunio Wanga2, Catriona Waitt3, Angela Colbers4, Helen Reynolds5, Nengjie He6, Lucy Read7, Mohammed Lamorde8, Saye Khoo9, Landon Myer10
1University of Cape Town, Cape Town, South Africa, 2 Desmond Tutu HIV Foundation, Cape Town, South Africa, 3University of Liverpool, Liverpool, United Kingdom, 4Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 5Infectious Diseases Institute, Kampala, Uganda, 6Radboud University Medical Center, Nijmegen, Netherlands, 7Liverpool School of Tropical Medicine, Liverpool, UK, 8Infectious Disease Institute, Kampala, Uganda

Background: There is extensive evidence of non-adherence in pregnant and postpartum women living with HIV (PPWH). But despite the expansion of dolutegravir (DTG) replacing efavirenz (EFV) in first-line ART, there are few data on objective adherence to DTG vs EFV and how non-adherence is associated with elevated viral load (VL) in this population.

Methods: The DoPHIN-2 trial (NCT0249181) randomized pregnant women initiating ART from 28w gestation to DTG vs EFV with tenofvir (TDF) and lamivudine/emtricitabine. Within the trial cohort we conducted a nested case-control study to examine adherence to DTG vs EFV using random plasma tenofovir (TFV) levels as an objective adherence measure in both arms (≥35.5ng/mL indicating effective adherence [EA]). Eligible participants had an initial VL>1000 at enrolment and achieved viral suppression (VS=≤20) during follow-up through 18m postpartum. Case specimens had ≥1 VL>20 after initial VS; control specimens were incidence density sampled from PHIV with persistent VS, matched on ART duration and trial arm (DTG vs EFV). Additional specimens were included from the suppressed visit preceding the first VL>20 (cases) or a time-matched visit (controls) and all visits after viremia for cases (subsequent visits). Logistic regression, with conditional models for matched data, was used to examine associations between EA and elevated VL.

Results: Overall 172 case and 338 control specimens were included from 88 PPWH (mean age, 28y). At preceding visits with EA, VS was higher in DTG compared to EFV (58% vs 42%). Self-reported missed doses (4%) and ARV-related side effects (2%) were low and similar by regimen. At the time of VL>20, cases had a mean VL 9.5log10 copies/mL; EA was observed in 37% of cases compared with 74% of controls with VL<20 at a matched ART duration. Differences were consistent between DTG (OR=6.3; 95% CI=2.3–17.2) and EFV (OR=3.8; 95% CI=1.3–10.8). Among cases, at the preceding visit 46% had EA compared with 37% at viremic visit (conditional OR=1.495 CI 7.2-7.6) with no difference by regimen. At subsequent visits, 86% with detectable TFV achieved VS again with no differences by regimen.

Conclusion: The association between objectively measured adherence and viremia was similar with DTG versus EFV. EA was higher in DTG at visits with VL>20. Taken together, these data suggest that DTG may be associated with better ART adherence compared to EFV but is not more forgiving of the short-term non-adherence that occurs commonly during the peripartum period.

906 A Novel Risk Predictor to Calculate Sub-Optimal Outcomes Among Pregnant and Postpartum Women With HIV
Karen Hampsland1, Kevin Owuor2, Laura K. Beres2, Emmah Ouma2, Mariannach A. Onono2, Anna Helvo2, Merecelline Onyando2, Jeff Szychowski2, I. Aboung3, Janet M. Turan4
1University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 2Kenyatta Medical Research Institute, KiM, Kenya, 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 4Kenyatta Medical Research Institute, Nairobi, Kenya, 5University of Alabama at Birmingham, Birmingham, AL, USA

Background: Pregnant and postpartum women living with HIV (PPWH) experience differential treatment success. No tool currently exists to assess a PPWH’s cumulative risk of disengagement from care or treatment failure with a quantifiable score during the peripartum period. To identify PPWH at risk and intervene before negative outcomes occur, this study sought to develop and validate a parsimonious risk calculator capable of predicting disengagement from care and treatment failure.

Methods: We used a derivation dataset with data from 1331 PPWH enrolled in the Mother-Infant Wildfire Adherence and Treatment Engagement trial in southwestern Kenya. Least absolute shrinkage and selection operator (LASSO) logistic regression procedures selected the most predictive variables from a list of 16 candidate factors based on prior research, including psychosocial, demographic, and clinical factors. We applied the Minimum Extended Bayes information criterion (EBIC) and 10-fold cross validation methods to find the regularization parameter lambda to give the minimum mean cross-validated
907 Mentor Mothers! Community Model to Attain Elimination of Mother-to-Child HIV Transmission, Tanzania

Neema E. Makyao, Amani Mare, Agnes Kosia, Aaron Godwin, Donatha Kayoza, Amos Nyirenda

1National Institute for Medical Research–Mbeya Medical Research Centre, Dar es Salaam, United Republic of Tanzania, 2Amani Health Africa, Dar es Salaam, United Republic of Tanzania, 3San Francisco Department of Public Health, San Francisco, CA, USA, 4Christian Social Services Commission, Dar es Salaam, United Republic of Tanzania

Background: Despite the remarkable progress in Tanzania on ART provision to pregnant women living with HIV there are still significant number of new HIV infections among children. Data shows that progress in preventing HIV transmission from mother to child has almost stagnated. These can be linked with poor uptake of HIV testing, gaps in ART initiation, low retention rates and poor adherence to treatment. Social cultural factors associated with early pregnancy stages can also be linked to the above challenges. We aim to address these gaps through the use of a community peer model accepted and trusted by the community members.

Methods: From January to June 2023 Amref implemented a community model using mentor mothers(MM) who are women living with HIV or breastfeeding champions identified from PMTCT clinics. Selection criteria included good adherence, willingness to disclose or have disclosed status and influential who can perform the task. We implemented it in 310 health facilities within 10 regions and 58 councils. A total of 947 MM who were trained, paired with Antenatal care supervisors in a health facility within their catchment areas were identified. Each facility selected a total of 4 MM and one facility supervisor who were trained using MM National curriculum.

Results: The implementation has increased early ANC bookings, couple counselling and HIV testing as well as increased number of women returned to care. From January to June 2023, we reached a total of 59,582 (93%) of pregnant women who were tested for HIV out of our target and 398 the positivity rate was 0.7%. We reached 51,818 women who tested for HIV. 540 were HIV+ which was 1.1%, their male partners reached were 36,888 and 395 were HIV positive. 254 clients who were lost to follow up and linked back to services. To ensure retention a total of 1,839 beneficiaries from 289 groups are continuously attended PMTCT were identified. Each facility selected a total of 4 MM and one facility supervisor who were trained using MM National curriculum.

Conclusion: Mentor mothers model has improved follow up and retention to care for BPFW through out the continuum of care, it is a community peer model accepted by the community and hence scale up will contribute to reaching eMTCT.

908 Comparison of Models of Care to Promote Postpartum Viral Suppression in South African Women

Mustafa Shuaib1, Tamini Philip1, Jasanthi Odayat2, Thokozile R. Malaba1, Elaine J. Abrams1, Landon Myer1

1University of Cape Town, Cape Town, South Africa, 2Columbia University, New York, NY, USA

Background: Maintaining viral suppression (VS) in postpartum women on antiretroviral therapy (ART) is a major concern. There is significant interest in both integrated models of service delivery for maternal and child health (MCH), and in differentiated service delivery models (DSD) postpartum, but there are few rigorous data comparing these intervention strategies.

Methods: We conducted a secondary analysis of individual patient trial data from Cape Town to compare head-to-head (i) an integrated MCH model with maternal and child care co-located and co-scheduled (MCHART; NCT01934777) versus (ii) a DSD model with mothers referred to community based “adherence clubs” for maternal ART dispensing (PACART; NCT0220054). Data for both interventions came from RCTs conducted in the same primary care community health service comparing each intervention to the local standard of care (SOC, referral to general adult ART services). Both trials measured demographic and behavioural covariates using the same tools; study viral load testing was conducted by trial personnel separate to routine antenatal ART (tenofovir+lamivudine+efavirenz). Analyses used frequentist network methods via generalised linear mixed models to compare VS (<50 copies/ml) under each model of care at 6 and 12 months postpartum using the SOC as the reference; results are reported as odds ratios (OR) with 95% CI.

Results: A total of 882 women (mean age: 29y; median time postpartum at enrolment, 1w (IQR, 0.6–1.9)) were included: 471 in MCHART and 411 in PACART. Follow-up through 12m was >85% in both trials; in women retained, VS was achieved by 299/375(80%) and 289/349(83%) women at 6m, and by 229/336(68%) and 231/329(70%) women at 12m, postpartum in MCHART and PACART, respectively. VS in integrated MCH at 6m and 12m was higher than in the DSD model (6m: 88% vs 87%; 12m: 80% vs 74%); VS under the SOC was higher in PACART than MCHART (6m: 79% vs 71%; 12m: 67% vs 55%, both p<0.05). In network comparisons, integrated MCH in MCHART was associated with significantly higher levels of VS compared to the DSD model in PACART at 12m postpartum (OR 2.48; 95%CI 1.25–4.95; Table); results were consistent at VL<1000 copies/ml and robust across a range of sensitivity analyses.

Conclusion: These novel data comparing two postpartum interventions in the same community suggest that integration of ART and postpartum MCH services achieved higher levels of VS compared to referral of mothers to DSD models of care in this setting.

909 Budget Impact Analysis of an Enhanced Retention Strategy for PMTCT Programs in Uganda

Elly Nuwamanya1, Mohammed Lamorde1, Ronald M. Galliwango1, Tabitha Ayabo1, Dianah Namuddu1, Benjamin C. Johnson2

1Infectious Diseases Institute, Kampala, Uganda, 2The Johns Hopkins Hospital, Baltimore, MD, USA

Background: Novel retention strategies have the potential to reduce mother-to-child transmission and improve patient outcomes for people living with HIV. The enhanced retention strategy (ERS) of the DoPHIN-2 trial in Uganda achieved a retention rate of 92% among women receiving PMTCT services and reduced perinatal transmission (PT) from 2.8% (national rate) to 1.5% (DoPHIN cohort). This analysis built on the DoPHIN findings by estimating the budget impact of the ERS compared to the standard of care (SOC) approach for preventing PT among women initiating antiretroviral therapy (ART) in late pregnancy in Uganda.

Methods: A budget impact analysis (BIA) was conducted from the payer (Uganda Ministry of Health) perspective with a 5-year time horizon. A
Microsoft Excel-based BIA model was populated with HIV epidemiological data and expenditures from the literature and the DolPHIN trial. These cost projections accounted for a variety of programmatic inputs, disease progression, differences in mortality based on treatment status, subsequent pregnancies, and other factors. The eligible population matched the DolPHIN inclusion criteria, including pregnant women with an adjustment for pre-pregnancy and ART data. We conservatively assumed a 50-50% market share for the ERS and SOC and an annual discount rate of 3%. Main outcomes of the analysis were incremental budget costs and infections averted over 5 years.

Results: Adapting the ERS would lead to a net cost increase of $64 million over the next 5 years, or a net cost increase of $13.8 million per year compared to the SOC. Newly enrolled patients account for $40 million of these marginal costs, while in-system patients account for $24 million. Direct programmatic costs of the ERS only account for 3% of this additional cost, with 97% of the marginal increase coming from the cost of providing ART for women who would otherwise be lost to follow-up. The ERS would avert an additional 6,933 infant infections compared to the SOC, and more than double the probability that women would be retained on ART at the start of subsequent pregnancies (24.94% for ERS vs 10.87% for SOC).

Conclusion: Implementing the ERS is likely to produce a significant budget impact to Uganda’s Ministry of Health while potentially offering substantial health benefits to people living with HIV. Though costly, the ERS gives an alternative to reducing loss-to-follow-up among marginalized groups.

910 Hypertension in Pregnant Persons by HIV Status and by DTG vs EFV Use in Botswana

Denise L. Jacobson1, Modiegi Diseko2, Judith Mabuta2, Ellen Caniglia1, Kathleen M. Powis3, Lynn Yee, Joseph M. Makhema, Shalim Lockman, Roger Shapiro, Rebecca Zash3, Jennifer Jao1, Elton Mukonda1, Landen Myer2, Landon Myer2, Jack Hu1, Jami Josefson4, Patrick Catalano5, Rebecca Zash3

1Northwestern University, Chicago, IL, USA, 2University of Cape Town, Cape Town, South Africa, 3ICAP at Columbia University, New York, NY, USA, 4Tufts University, Boston, MA, USA, 5Botswana Harvard AIDS Institute Partnership, Gabone, Botswana, University of Pennsylvania, Philadelphia, PA, USA, 6Massachusetts General Hospital, Boston, MA, USA, 7Northwestern University Feinberg School of Medicine, Chicago, IL, USA, 8Binghamton University, Binghamton, NY, USA, 9Beth Israel Deaconess Medical Center, Boston, MA, USA

Background: In non-pregnant adults, some studies suggest dolutegravir (DTG) is associated with increased risk of hypertension (HTN). Given the serious implications of HTN for pregnancy outcomes, we compared the risk of hypertensive disorders of pregnancy (HDP) among pregnant people on DTG-based antiretroviral treatment (ART) to those on efavirenz (EFV)-based ART and to pregnant women without HIV (w/o HIV).

Methods: Methods: Among deliveries captured by the Tsepamo Birth Outcomes Surveillance Study (8/2014-8/2022), we included people who presented to antenatal care prior to 20 weeks gestational age (GA) and were either w/o HIV or with HIV and conceived 0.5-5 years after starting DTG- or EFV-based ART.

Blood pressures (BP) and medical history of HTN were abstracted from antenatal medical records. Chronic HTN was defined as a pre-pregnancy history of HTN or HTN (systolic BP >140 or diastolic BP >90 mm Hg) before 20 weeks GA. HDP was onset of any HTN (including mild and severe) >20 weeks and predelivery among women without chronic HTN. We determined proportions with chronic HTN and other factors. The eligible population matched the DolPHIN inclusion criteria, including pregnant women with an adjustment for pre-pregnancy and subsequent pregnancies, and other factors. The eligible population matched the DolPHIN inclusion criteria, including pregnant women with an adjustment for pre-pregnancy and ART data. We conservatively assumed a 50-50% market share for the ERS and SOC and an annual discount rate of 3%. Main outcomes of the analysis were incremental budget costs and infections averted over 5 years.

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911 Gestational Diabetes in South African Women With HIV on Dolutegravir: Results From the ORCHID Study

Jennifer Jao1, Elton Mukonda1, Landen Myer2, Elaine J. Abrams1, Hiwinge Madlala1, Jack Hu1, Jami Josefson4, Patty Catalano5, Jack Hu1, Jami Josefson4, Patrick Catalano5

1Northwestern University, Chicago, IL, USA, 2University of Cape Town, Cape Town, South Africa, 3ICAP at Columbia University, New York, NY, USA, 4Tufts University, Boston, MA, USA

Background: Few data exist on gestational diabetes (GDM) in Africa, particularly in women with HIV (WWH) receiving dolutegravir-based antiretroviral therapy (ART). This study aimed to assess the association of HIV infection and duration on tenofovir/lamivudine/dolutegravir (TDL) with GDM.

Methods: The ORCHID study enrolls WWH initiating/receiving TDL and HIV-seronegative (HIV-) women >16 years and <18 weeks gestational age (GA) in South Africa. Pregnant women with diabetes or hypertension are excluded. Participants undergo air displacement plethysmography assessment of body composition and a 75g oral glucose tolerance test at enrollment and 32-34 wks GA.

Results: Of 265,410 deliveries in the study period, we included 127,946; 5,866 women without chronic HTN. We determined proportions with chronic HTN and other factors. The eligible population matched the DolPHIN inclusion criteria, including pregnant women with an adjustment for pre-pregnancy and subsequent pregnancies, and other factors. The eligible population matched the DolPHIN inclusion criteria, including pregnant women with an adjustment for pre-pregnancy and ART data. We conservatively assumed a 50-50% market share for the ERS and SOC and an annual discount rate of 3%. Main outcomes of the analysis were incremental budget costs and infections averted over 5 years.

Results: Of 265,410 deliveries in the study period, we included 127,946; 5,866 conceiving on DTG, 4,771 conceiving on EFV, and 117,309 w/o HIV. Median maternal age was 25 yrs in those w/o HIV and 31 yrs in the DTG and EFV groups (Table 1). The prevalence of chronic HTN was 4.4%, 4.4% and 4.6% and the risk of HDP was 10.2%, 8.1% and 11.7% in the DTG, EFV and w/o HIV groups, respectively. The risk of HDP was 20% lower (ARR=0.80, 95% CI 0.71,0.91) in the EFV group and 20% higher (ARR=1.20, 95% CI 1.10,1.30) in the w/o HIV group, compared to the DTG group.

Conclusion: The prevalence of chronic HTN was similar across exposure groups. While pregnant women who conceived on DTG-based ART had a higher risk of HDP than those on an EFV-based regimen, both groups had a lower risk than those without HIV. In future analyses it will be important to elucidate the mechanisms responsible for these differences, including the impact of weight and weight gain.

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912 Pregnancy History Affects Age-Related Comorbidity Burden in US Women With and Without HIV
Lauren F. Collins1, Cyra C. Mehta2, Ava Cox1, Qian Yang1, Tina Tisdale1, Martina Badell3, Igbo Oftokun1, Daniel Westreich4, Adaoa Adimora5, Seble Kassaye1, Elizabeth F. Toppert1, Deborah Konkle-Parker1, Aadhia Rana1, Maria L. Alcalde6, Anandi N. Sheth7
1Emory University, Atlanta, GA, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 3George Washington University, Washington, DC, USA, 4The Johns Hopkins University, Baltimore, MD, USA, 5University of Mississippi Medical Center, Jackson, MS, USA, 6University of Alabama at Birmingham, Birmingham, AL, USA, 7University of Miami, Miami, FL, USA

Background: Despite non-AIDS comorbidities (NACM) being more common and occurring earlier in life among women versus men with HIV, evidence is lacking to understand potential drivers of sex differences. We evaluated the effect of reproductive history on NACM development in women.

Methods: We performed a cross-sectional analysis of the Study of Treatment And Reproductive outcomes (STAR), a longitudinal cohort of women with and without HIV (WWH; WwoH) aged 18-45 years enrolled in 6 Southern U.S. sites. NACM prevalence and burden (total NACM count of 12 assessed) was determined at STAR enrollment. Pregnancy history was categorized as zero, 1-2, or ≥3 pregnancies. Prior linear regression models evaluated the association of NACM burden with HIV serostatus, age, and pregnancy history.

Results: Among 519 women (354 WWH; 165 WwoH), median age was 36 (Q1-Q3 30-41) years, 75% reported Black race, 45% ever smoked; 22%, 32%, and 46% had zero, 1-2, and ≥3 pregnancies, respectively. Among WWH, median CD4 count was 666 (Q1-Q3 448-938) cells/mm³ and 77% had HIV-1 RNA < 200 c/ml. NACM prevalence was (WWH:WwoH): obesity (59%/55%), psychiatric illness (54%/46%), diabetes (38%/30%), hypertension (23%/26%), bone disease (25%/28%), arthritis (8%/6%), cardiovascular disease (7%/4%), liver disease (7%/1%), dyslipidemia (4%/3%), kidney disease (3%/1%), non-AIDS cancer (1%/1%). Among women with available data for all NACM assessed (n=332), WWH vs WwoH had a mean NACM burden of 2.5 vs 2.4, p=0.24. Among women overall, mean NACM burden increased with age group: 1.9 (18-24y), 2.2 (25-29y), 2.5 (30-34y), 2.7 (35-39y), 2.7 (40-45y) (p-trend=0.002). Among women with zero, 1-2, ≥3 pregnancies, age-adjusted mean NACM burden was 2.4, 2.2, and 2.6 (p-trend=0.20). HIV serostatus did not modify the effect of age and pregnancy history on NACM burden (HIV*a*age*pregnancy interaction p=0.76). Among women across HIV status, pregnancy history was associated with estimated NACM burden in certain age groups: 18-24y (p-trend=0.09), 25-29y (p-trend=0.03), 30-34y (p-trend=0.11), 35-39y (p-trend=0.21), 40-45y (p-trend=0.71) (Figure).

Conclusion: Among reproductive age women in the U.S. South, the burden of 12 NACM was high overall, increased with age, and was associated with pregnancy history in some age groups; further, the distribution of NACM prevalence differed by HIV serostatus. These data may inform the development and timing of NACM screening and prevention strategies to be deployed across the reproductive life course.
915 Changes in Body Composition During Pregnancy in South African Women Living with HIV on ART

Hiengiwe P. Madlala1, Lara Dugas1, Jennifer Iao1, Elaine J. Abrams1, Elton Mukonda1, Hayli Geffen1, Julia Goedecke1, Patrick Catalano1, Grace A. McComsey1, Allison Zerbe1, Justine Legbedze2, Landon Myer2

1University of Cape Town, Cape Town, South Africa, 2Northwestern University, Chicago, IL, USA

Methods: In the Obesogenic Origins of Maternal and Child Metabolic Health Involving Dolutegravir (ORCHID) study, we explored the relationship between HIV and ART duration and changes in body composition (weight, fat mass (FM), fat-free mass (FFM)) between ≤18 weeks (T1) and 32-34 weeks (T3) of pregnancy. We enrolled WLH receiving tenofovir/lamivudine/dolutegravir (TLD) and HIV-seronegative (HIV-) women (enrollment age ≥16y and ≤18y gestational age (GA)). Weight, FM and FFM were measured using air displacement plethysmography adjusted for the increase in hydration constant of FFM at T3; resting energy exposure (REE) was measured using indirect calorimetry; GA was confirmed via ultrasound; physical activity was estimated using the Pregnancy Physical Activity Questionnaire. Linear regression was used to explore the relationship between HIV status and TLD duration (<28d, 28-182d and >182d) with changes in body composition parameters between T1 and T3 after adjusting for potential confounders.

Results: Overall 970 women were followed: 376 WLH (80% VL <50 copies/mL; 92% CD4 >200 cells/µL; median duration TLD 126d (IQR, 12-465) and 594 HIV- women; for weight and FM these reductions were greatest in WLH on ART. The absence of any association was not altered in subgroups of women with BMI<30kg/m² or in women with their first BP measures <18 wks gestational age (GA) in Cape Town, South Africa. Women with prevalent diabetes or hypertension were excluded. All WLH were on tenofovir-lamivudine-dolutegravir (TLD). Women were followed through 6 weeks postpartum with serial, standardised BP measures (three measures of the left arm using an automated, calibrated BP cuff sized to participant BMI) conducted between September 2021 and September 2023, 1601 women were enrolled (633 WLH, 968 HIV-; median (IQR) age 28 years (24–32); BMI 30 kg/m² [25–35]; GA at enrolment 13 weeks [10-16]; 32% primigravida; 6% current smokers). In WLH median duration of TLD use was 190 days (IQR 115-330) and 30% of women initiated TLD in the preceding month. Mean sBP and dBP in pregnancy were similar throughout pregnancy until 6 weeks postpartum between WLH and HIV- controls (Figure). This absence of any association was not altered by adjustment for maternal age, GA, BMI, smoking or family history (sBP coefficient, -0.39mmHg; 95%CI: -1.33, 0.58; dBP coefficient, 0.13mmHg; 95%CI: -0.59, 0.84). In separate analyses increasing duration of TLD was not associated with changes in sBP or dBP (not shown). Through 6 weeks postpartum 9% of women experienced incident hypertension, with no variation by HIV status (adjusted hazard ratio, 1.27; 95% CI, 0.80, 1.94) or duration of DTG use among WLH. The absence of any association was not altered in subgroups of women with BMI<30kg/m² nor in women with their first BP measures <18 weeks GA. In adjusted models, higher sBP/dBP was associated with increasing age and BMI.

Conclusion: While longer durations of follow-up are required to understand the cardiovascular health of WLH on TLD, these reassuring data suggest no association during pregnancy of HIV status or TLD duration with BP or incident hypertension.

916 Association of HIV and Dolutegravir With Changes in Blood Pressure During Pregnancy and Postpartum

Landon Myer1, Elaine J. Abrams2, Mustafa Shuaib3, Hiengiwe P. Madlala3, Sandisiwe Matyseni1, Phindi Zwane1, Allison Zerbe4, Jennifer Iao5

1University of Cape Town, Cape Town, South Africa, 2Columbia University, New York, NY, USA, 3ICAP at Columbia University, New York, NY, USA, "Ann & Robert H Lurie Children’s Hospital of Chicago, Chicago, IL, USA, 4Northwestern University, Chicago, IL, USA

Background: There is growing interest in whether dolutegravir (DTG) use may be associated with changes in blood pressure (BP) in women living with HIV (WLH) but there are few data from pregnant and postpartum women, in particular with BP measures from early pregnancy, and few comparisons with women testing HIV-.

Methods: Within the Obesogenic Origins of Maternal and Child Metabolic Health with Dolutegravir (ORCHID) cohort, we enrolled 1601 women at ≤18 wks gestational age (GA) in Cape Town, South Africa. Women with prevalent diabetes or hypertension were excluded. All WLH were on tenofovir-lamivudine-dolutegravir (TLD). Women were followed through 6 weeks postpartum with serial, standardised BP measures (three measures of the left arm using an automated, calibrated BP cuff sized to participant BMI) conducted between September 2021 and September 2023, 1601 women were enrolled (633 WLH, 968 HIV-; median (IQR) age 28 years (24–32); BMI 30 kg/m² [25–35]; GA at enrolment 13 weeks [10-16]; 32% primigravida; 6% current smokers). In WLH median duration of TLD use was 190 days (IQR 115-330) and 30% of women initiated TLD in the preceding month. Mean sBP and dBP in pregnancy were similar throughout pregnancy until 6 weeks postpartum between WLH and HIV- controls (Figure). This absence of any association was not altered by adjustment for maternal age, GA, BMI, smoking or family history (sBP coefficient, -0.39mmHg; 95%CI: -1.33, 0.58; dBP coefficient, 0.13mmHg; 95%CI: -0.59, 0.84). In separate analyses increasing duration of TLD was not associated with changes in sBP or dBP (not shown). Through 6 weeks postpartum 9% of women experienced incident hypertension, with no variation by HIV status (adjusted hazard ratio, 1.27; 95% CI, 0.80, 1.94) or duration of DTG use among WLH. The absence of any association was not altered in subgroups of women with BMI<30kg/m² nor in women with their first BP measures <18 weeks GA. In adjusted models, higher sBP/dBP was associated with increasing age and BMI.

Conclusion: While longer durations of follow-up are required to understand the cardiovascular health of WLH on TLD, these reassuring data suggest no association during pregnancy of HIV status or TLD duration with BP or incident hypertension.
917 High Proportions of Adverse Births in Women With HIV and Non-Communicable Disease Comorbidities

Amohelang Lehmola, Emma Kalk, Mary-Ann Davies, Dorothy Nyemba, Ushma Mehta, Thokozile R. Malaba, Gregory Petro, Andrew Boule, Landon Myer, Hlengiwhe P. Madlala
University of Cape Town, Cape Town, South Africa

Background: HIV/antiretroviral therapy (ART) and non-communicable diseases (NCDs) like hypertension, diabetes and obesity are independently implicated in poor pregnancy outcomes. However, there is limited data on the interplay of HIV/ART and these NCDs, and associations with adverse birth outcomes in South African women.

Methods: In a retrospective study in an urban primary care antenatal care facility (ANC) in Cape Town, South Africa, 470 women living with HIV (WLH) and 505 without HIV (HIV-) ≥18 years) were enrolled from the first ANC visit between 2017 and 2019 and followed through to delivery. We examined HIV, hypertension (HPT), diabetes mellitus (DM) alone and HIV with obesity, hypertension, and diabetes co-morbidity (irrespective of chronicity) and the following outcomes: preterm delivery (PTD <37 gestational weeks), low birthweight (LBW <2500g), high birthweight (HBW >4000g) (abstracted from medical records) and small for gestational age (SGA <10 percentile), large for gestational age (LGA >90th percentile) (generated using the INTERGROWTH tool). Differences in proportions of adverse birth outcomes between exposure groups were tested with Chi-squared tests among live singleton births.

Results: In this study, median age was 29y (IQR,25-33) and 21% of women were primigravid. Additionally, 47% were obese (BMI≥30 kg/m²), 8% hypertensive and 2% diabetic. Overall, 10% of infants were PTD, 11% LBW, 4% HBW, 10% SGA and 10% LGA. Women with HPT only had 41% PTD, 35% LBW and 29% SGA. Those with obesity only had 4% PTD, 5% LBW, 6% HBW, 5% SGA and 17% LGA. Excluding all NCDs, WLH had 11% PTD, 14% LBW, 3% HBW, 14% SGA and 7% LGA. WLH with obesity had higher LGA (12 vs 7%, p <0.01) but lower PTD (6 vs 11%, p <0.01), LBW (7 vs 14%, p <0.01) and SGA (5 vs 14%, p <0.01) compared to WLH only. WLH with hypertension co-morbidity had higher PTD (22 vs 11%, p = 0.01), LBW (22 vs 14%, p = 0.03) and SGA (22 vs 15%, p = 0.03) compared to WLH only. Further, WLH with both obesity and HPT had higher LBW (27 vs 11%, p = 0.04) and LBW (19 vs 14%, p = 0.03) compared to WLH only. DM only coexisted with other co-morbidities and not HIV.

Conclusion: WLH and an NCD co-morbidity had a higher proportion of some adverse birth outcomes compared to WLH only. Integration of NCD management interventions with ANC services is essential to avert excess adverse outcomes in high HIV burden settings.

918 DTG Versus EFV Initiation in Pregnancy Is Not Associated With Postpartum Blood Pressure

Thokozile R. Malaba; Sylvia Nasiwa; Nengjie He; Helen Reynolds; Jim Read; Lucy Read; Catherine Orrell; Angela Colbers; Catriona Waitt; Mohammed Lamorde; Saye Khoo; Duolao Wang; Landon Myer; for the DolPHIN-2 Study Group

1 University of Cape Town, Cape Town, South Africa, 2Infectious Disease Institute, Kampala, Uganda, 3Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 4University of Liverpool, Liverpool, United Kingdom, 5Desmond Tutu HIV Foundation, Cape Town, South Africa, 6Radboud University Medical Center, Nijmegen, Netherlands

Background: Several studies in non-pregnant adults have suggested increases in blood pressure (BP) associated with dolutegravir (DTG) use, however findings are mixed. There are notably few data from i) sub-Saharan Africa and ii) pregnant and postpartum women living with HIV (PLH).

Methods: We compared BP in PLH initiating DTG- versus Efavirenz-(EFV-)- containing regimens initiated during pregnancy in a secondary analysis of the DolPHIN-2 trial (NCT03249181). At sites in Uganda and South Africa, PLH initiating ART ≥28w gestation were randomly assigned tenofovir + lamivudine/ emtricitabine and either DTG or Efavirenz (EFV) as first-line therapy. PLH were followed with 8 study visits through 72 weeks postpartum including standardised anthropometric and BP assessments including sized cuffs. Given physiologic changes in BP during the perinatal period, analyses focused on systolic (sBP) and diastolic BP (dBP) at 24, 48 and 72 weeks postpartum in PLH assigned to DTG vs EFV using mixed effects linear models adjusted for age, BMI, site, and enrolment BP; temporal changes were evaluated using time interactions in separate models. In addition, we examined the risk of incident hypertension (BP >140/>90) among those with BP <140/>90 at enrolment and 6 weeks postpartum.

Results: Overall 268 women were enrolled (median age 28 years; median gestation 31 weeks; median BMI 28kg/m²). Participants in South Africa had consistently higher BMI, sBP and dBP compared to those in Uganda at enrolment and throughout follow-up. However after accounting for site and baseline values there were no associations observed between DTG use and sBP (beta=2.2mmHg; 95% CI: -0.6 to 5.0), dBP (beta=1.54mmHg; 95% CI: -0.9 to 2.3) and weight (beta=0.7kg; 95% CI: -0.9 to 2.3) through 72w postpartum. Four participants had hypertension detected at enrolment; during follow-up 8 participants switched treatment assignment, none related to blood pressure or weight gain. There was no association between DTG versus EFV and incident hypertension at any time point.

Conclusion: These reassuring RCT data suggest that after adjustment for important pre-treatment covariates there was no association between DTG vs EFV initiated late in pregnancy and BP through 18 months postpartum. There is ongoing need for attention to the long-term cardiometabolic effects of DTG use in PLH.
919 The Impact of HIV and the Postpartum Period on the Gut Microbiota in South African Women

Lara R. Dugas1, Hiengpiwe P. Madlala1, Gertrude Eklou-Mensah1, Candice Coo-Kang1, Julia Goedecke1, Amy Mendenham1, Jess Davies1, Chad Africa1, Demi Meyer1, Jack Gilbert1, Brian Layder1, Jennifer Jiao1, Elaine J. Abrams1, Angela Bengtson1, Landon Myer1

1University of Cape Town, Cape Town, South Africa; University of California San Diego, La Jolla, CA, USA; Loyola University Chicago, Chicago, IL, USA; South African Medical Research Council, Cape Town, South Africa; South Australia Health, Lympol, Australia; University of Illinois at Chicago, Chicago, IL, USA; Northwestern University, Chicago, IL, USA; Columbia University, New York, NY, USA; Emory University, Atlanta, GA, USA

Background: The perinatal and post-partum (PP) period is a window to future metabolic health, including type 2 diabetes (T2D). Between the 1st and 3rd trimester (T3), the gut microbiota (GM) is altered and associated with increased inflammation and reduced insulin sensitivity (IS) at T3. Similarly, among persons with HIV (PWH), there is emerging evidence for GM alterations, including reduced microbial diversity and altered functional features. Approximately 30% of pregnant South African (SA) women are PWH. We hypothesized that the composition of the GM differed by HIV status and PP period among SA women.

Methods: We performed a cross-sectional analysis of GM composition, using 16S rRNA amplicon sequencing, on early morning stool samples collected from 65 PP women originally enrolled in the Cardiometabolic Health in Pregnancy study (2019-2022). Body composition was measured using dual x-ray absorptiometry and IS measured using an oral glucose tolerance test to derive the Matsuda index.

Results: Of 65 PP women, 46 were PWH, with no differences in the length of the PP period by HIV status. PWH had lower BMIs (28.1 [24.1-33.8] vs. 31.2 [27.8-40.2] kg/m2, p=0.046), and fat (31.9 [25.1-43.3] vs. 40.3 [27.5-54.5] kg) and fat free mass (37.4 [34.6-43.6] vs. 42.6 [36.9-49.0] kg), but similar fastest glucose, insulin, and Matsuda Index. After demultiplexing, 2,162,496 sequence reads were obtained from the stool samples, with a median of 32,990 sequence reads. Overall, 1,569 amplicon sequence variants (ASVs) including 19 phyla and 370 genera were identified, with the most abundant phyla across all the two groups being Firmicutes, Bacteroidetes, Firmicutes C and D and Proteobacteria. The PP period also differed by microbial taxa, whereby those > 12 months had a greater proportion of Ellagibacter, associated with anti-inflammatory activity.

Conclusion: We confirm that the GM differs by HIV status and the length of PP period. Future research should explore the persistent effects of the PP period and HIV status on the composition of the GM and the role of inflammation and IS in SA.

920 A Multicomponent Intervention Improves Disclosure and ART Adherence Among Pregnant and Postpartum Women

Jane Kabami1, Laura B. Balzer1, Faith Kapoya1, Jaffir Okiring1, Joannita Nangendo1, Emmanuel Ruhayamanyaka1, Peter Ssebutinde1, Elizabeth Arinitwe1, Michael Auyai2, Stella Kabage1, Anne R. Kataho1re, Moses R. Kamya4, Philippa Musoke1, Dinah Ramaabya1, Francis Randa1, Alexander C. Tsai1, Betsy Kammere1, Adam R. Cassidy5, Elizabeth Arinitwe1, Angela Bengtson1, Brian Layder1, Jennifer Jiao1, Elaine J. Abrams1, Angela Bengtson1, Landon Myer1

1Infectious Diseases Research Collaboration, Kampala, Uganda, University of California Berkeley, Berkeley, CA, USA, 2Utkale University, Durban, NC, USA, 3Mbarara District Health Office, Mbarara, Uganda, 4Makerere University College of Health Sciences, Kampala, Uganda

Background: Disclosure of HIV status and adherence to antiretroviral treatment (ART) among pregnant and postpartum women are critical for sustained HIV care engagement and elimination of vertical transmission in Sub-Saharan Africa. We evaluated the effect of a multi-component intervention, including Enhanced viral load (VL) counseling and Standardized Peer-mother Support (ENHANCED-SPS), on disclosure and ART adherence among pregnant and postpartum women with HIV in rural Uganda.

Methods: We developed an ENHANCED-SPS intervention informed by the empirically-validated PRECEDE framework. Intervention components included: 1) Provider training and mentorship on enhanced VL counselling (predisposing), 2) standardized peer mother support and bi-weekly phone calls to mothers to provide VL and adherence counselling (enabling) and, 3) point of care VL monitoring and feedback meetings with providers and peer mothers (reinforcing). At routine clinic visits, mothers also received an assessment of barriers to disclosure and adherence as well as discussion on plans to address those barriers. We evaluated the effect of the intervention on disclosure and adherence after 12-month of follow-up.

Results: We enrolled 505 pregnant and post-partum women at the 7 public health clinics from September 2019 to October 2020. Participants’ median age was 28 years (Q1:24, Q3:32), 157/505 (31%) were newly diagnosed with HIV, 318/455 (70%) were virally suppressed (HIV RNA<1000 c/ml) and 79% (95%CI:69-90%) disclosed their HIV status to anyone at baseline. After 12 months of the intervention, disclosure increased to 88% (95%CI:83-94%), corresponding to a 9% (95%CI:1-18%; p=0.002) absolute increase from baseline. The intervention increased disclosure within subgroups of age and enrollment group, especially among younger women (15-24years) with 17% increase (95%CI:0.5-29%; p=0.008) and the newly diagnosed pregnant women with 39% increase (95%CI:18-61%; p=0.003). Similar effects were observed when examining adherence to a partner or spouse. Additionally, the intervention improved adherence to ART to 93% (95%CI:83-100%), corresponding to a 25% (95%CI:12-39%; p=0.002) absolute increase from the baseline measurement of 68% (95%CI:62-73%).

Conclusion: The multi-component, peer-led, enhanced VL counselling intervention significantly increased disclosure of HIV status and ART adherence among pregnant and postpartum women within 1 year of implementation. Young women and 1st presentations with new HIV diagnosis had increased benefit.

921 High Prevalence of Depression and Anxiety in Women Without HIV and Women With HIV on DTG-ART

Kebaipe Moabi1, Gloria K. Mayindo1, Allison LeMahie1, Paige L. Williams1, Naledi Kamanga1, Ame Diphok1, Kathleen M. Powis1, Gaeselvile Matheto1, Dinh Ramaaby1, Francis Randa1, Alexander C. Tsai1, Betsy Kammere1, Adam R. Cassidy5, Elizabeth Arinitwe1, Angela Bengtson1, Brian Layder1, Jennifer Jiao1, Elaine J. Abrams1, Angela Bengtson1, Landon Myer1

1Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 2Mayo Clinic, Rochester, MN, USA, 3Harvard University, Cambridge, MA, USA, 4Massachusetts General Hospital, Boston, MA, USA, 5Botswana Ministry of Health, Gaborone, Botswana, University of Botswana, Gaborone, Botswana, 6Boston Medical School, Boston, MA, USA, 7Boston Children’s Hospital, Boston, MA, USA, 8University of Botswana, Gaborone, Botswana

Background: Common mental disorders are a leading global cause of disability. Little is known about the prevalence and nature of depression and anxiety symptoms among women living with HIV taking dolutegravir (DTG)-based antiretroviral treatment (ART), particularly in southern Africa.

Methods: From March 2021 to May 2022 (a period affected by COVID-19), we enrolled women living with HIV (most on DTG-based ART at enrollment) and without HIV in the “Mother” study of child neurodevelopment and maternal mental health, in one city and one village in Botswana. At enrollment, trained staff administered the Patient Health Questionnaire (PHQ-9) and Generalized Anxiety Disorder (GAD-7) in Setswana or English. PHQ-9 score >8 was defined as probable depression (per prior Botswana data) and GAD-7 >7 >8 as probable anxiety. For 3-category ordinal endpoints, we used PHQ-9 score 1-4 (minimal), 5-9 (moderate), and >9 (moderate/severe) for depression; and GAD-7 score 0-4
924 Metabolomic Perturbations of Tryptophan & Arginine Metabolites in the Breast Milk of Women With HIV

Nicole H. Tobin1, Fan Li1, Kathie G. Ferbas1, John W. Sleasman2, Louise Kuhn3, Grace M. Aldrovandi3

1University of California Los Angeles, Los Angeles, CA, USA, 2Duke University, Durham, NC, USA, 3Columbia University Medical Center, New York, NY, USA

Background: Infants born to women with HIV (WwH), but not infected (HIV-exposed uninfected, HEU) have two to three times the mortality rate of infants born to women without HIV (WwOH). Tryptophan is an essential amino acid critical for immune development, neurocognitive development, and growth. We investigated the milk metabolome from WwH and WWoH in the pre-ART era to determine if metabolic perturbations may contribute to the impaired immune development of infants born to WwH.

Methods: Untargeted metabolomics was performed on 1599 breast milk samples collected longitudinally up to 24 months post-partum from 38 WWoH and 288 WwH with known infant outcomes from a randomized clinical trial conducted in Lusaka, Zambia 2001-2008. The milks of WWH were further separated by infant outcome into 4 groups: HEU infants who survived (n=76) and 288 WwH with known infant outcomes from a randomized clinical trial conducted in Lusaka, Zambia 2001-2008. The milks of WWH were further separated by infant outcome into 4 groups: HEU infants who survived (n=76) and 288 WwH with known infant outcomes from a randomized clinical trial conducted in Lusaka, Zambia 2001-2008.

Results: Among 268 women median (IQR) EPDS scores were 8 (3-11) and highest at enrollment at the time of initial HIV diagnosis. In the dolutegravir-and efavirenz arm, respectively. 23.7% and 25.6% had an EPDS score above 9, indicating possible or probable depression. HADS scores were also highest at enrollment and decreased over time. An abnormal HADS score (above 11) was seen at least once during follow up in 42 of 49 patients (15.7%), although no differences were seen between treatment arms. For the anxiety-component 1.9% and 1.5% of the dolutegravir and efavirenz arms, respectively, had a score of 11 or higher. No association was found between EPDS, suicidality (question 10 of EPDS) and HADS scores and the assigned regimen (p = 0.93, 0.97 and 0.18 respectively). Abnormal scores for the depression-component were seen in 3.4% of participants in both treatment arms. Median (IQR) PSQI scores for dolutegravir- and efavirenz were 6 (5-7) and 5 (5-6.5) respectively, p=0.70.

Conclusion: No statistically significant differences were observed across these measures between pregnant and post-partum women treated with efavirenz- or dolutegravir containing regimens. Rates of depression on the EPDS were high, but decreased over the course of time and confirm the need for psychological support after initial HIV diagnosis in pregnancy.
were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate method.

**Results:** 1597/1599 samples were successfully analyzed with identification of 765 known biochemicals and 74 unknown biochemicals. Tryptophan levels were significantly lower in the milk samples of WWH compared to WWoH at all timepoints. Log-transformed kyurenine:tryptophan (KT) ratios were elevated in the milk of WWH at all timepoints (p < 0.001). The KT-ratio at 1 month was correlated with baseline maternal plasma and 1 month breast milk HIV RNA (r² of 0.28 and 0.20, respectively), and inversely with baseline CD4 count (r² = -0.32); all p < 0.001. Dimethylarginines was elevated in the milk of WWH during the first 9 months of lactation. A novel metabolite, X-12127, was significantly elevated in the milk of WWH at all timepoints following the first week of life (p < 0.001 through 12 mos, then p < 0.1 at 15 and 18 mos).

**Conclusion:** Tryptophan is significantly lower in the milk of WWH throughout early infancy. As breast milk serves as the only source of this essential amino acid, this depletion likely contributes to the impaired immune development of children born to WWH. Elevations of dimethylarginines may alter nitric oxide levels. Identifying key alterations and novel therapeutics is of critical importance for the one million children born to WWH each year.

**Figure:** Tryptophan levels are decreased and KT ratios are increased in the breast milk of WWH. A) Volcano plot from 1 month of lactation showing metabolites either decreased (blue) or increased (red) in milks of WWH. B) Boxplots of the milk KT ratio over the course of lactation.

**925 Breast Milk Transfer and Infant Exposures to DTG, TAF, and TFV: Results From IMPAACT 2010/VESTED**

Tk Nguyen¹, Jung-Woo Chae², Lauren Ziemba³, Anne Coletti⁴, Kevin Knowles⁵, Benjamin Johnston⁶, Patrick Jean-Philippe⁷, Tsongu Mhembere⁸, Tarin Chawana⁹, Dee Wabwire⁹, Violet Korutaro⁹, Shahin Lockman⁵, Maxensia Owor⁶, Lynda Stranix-Chibanda⁷, Dhayendre Moodley⁵, Avy Violari⁵, Brenda Kakayi⁵, Mary G. Fowler⁸, for the PROMISE 1077 Study Team

¹Makerere University—Johns Hopkins University Research Collaboration, Kampala, Uganda, ²Harvard TH Chan School of Public Health, Boston, MA, USA, ³University of Washington, Seattle, WA, USA, ⁴University of California San Diego, San Diego, CA, USA, ⁵University of Washington, Seattle, WA, USA, ⁶University of California San Diego, San Diego, CA, USA, ⁷University of California, Los Angeles, Los Angeles, CA, USA, ⁸University of Washington, Seattle, WA, USA, ⁹University of the Witwatersrand, Johannesburg, South Africa, ¹⁰Johns Hopkins University, Baltimore, MD, USA

**Background:** Recent studies observed detectable HIV-1 virus levels in breastmilk (BM) despite undetectable HIV-1 RNA viral load (VL) in plasma. This discordance between HIV VL in plasma and BM could account for residual vertical HIV-1 transmission during lactation. We assessed the association of vertical HIV-1 transmission with HIV VL in plasma and BM, and with tenofovir (TFV) drug levels.

**Methods:** This case-control study was nested within the IMPAACT PROMISE 1077BF perinatal HIV trial which evaluated maternal ARV strategies to prevent BM HIV transmission. Cases were mother-infant pairs with infants who had a positive HIV nucleic acid test (NAT) during the breastfeeding period; control pairs had infants who were HIV NAT negative. Two controls were matched for each case by infant sex, study site, maternal age at delivery, and 1077BF component at infection. Maternal plasma and BM collected near an infant’s infection date were assayed for HIV total nucleic acid (TNA; DNA + RNA) VL, DNA VL, RNA VL, and TFV concentration. Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs).

**Results:** 93 mother-infant pairs (31 cases; 62 controls) were enrolled from Malawi, Uganda, South Africa, Zimbabwe and India. Median maternal age at delivery was 25 years, 39 (42%) were male infants, 57 (61%) were randomized in the postpartum component. Median (Q1, Q3) age of infection was 6 (3, 14) months. Over 70% (22/31) of samples were taken on the same day or within one month of infection. Median (Q1, Q3) maternal plasma VL was 39,228 (4822, 124,886) copies/ml for cases vs 20 (20, 210) for controls. BM RNA VL was above lower limit of quantification for 17 (55%) cases and 7 (11%) controls. The odds of infant HIV infection were 2.6 times higher for each log increase in maternal plasma RNA VL (95% CI: 1.6-4.5) and 1.8 times higher for each log increase in BM RNA VL (95% CI: 1.3-2.3).

**Figure:** DTG, TAF, and TFV Concentrations in Breast Milk and Infant Plasma

**926 1077BF: Breast Milk Reservoir, Tenofovir Levels, and HIV Transmission Among Breastfeeding Mothers**

Maxensia Owor¹, Patricia DeMarrais², Kristin Baltrusaitis³, Lisa Frenkel⁴, Brookie Best⁵, Lynda Stranix-Chibanda⁷, Dhayendre Moodley⁵, Avy Violari⁵, Brenda Kakayi⁵, Mary G. Fowler⁸, for the PROMISE 1077 Study Team

¹Makerere University—Johns Hopkins University Research Collaboration, Kampala, Uganda, ²Harvard TH Chan School of Public Health, Boston, MA, USA, ³University of Washington, Seattle, WA, USA, ⁴University of California San Diego, San Diego, CA, USA, ⁵University of California, Los Angeles, Los Angeles, CA, USA, ⁶University of Washington, Seattle, WA, USA, ⁷University of the Witwatersrand, Johannesburg, South Africa, ⁸Johns Hopkins University, Baltimore, MD, USA

**Background:** Limited information is available on breast milk transfer and subsequent infant systemic exposure to dolutegravir (DTG), tenofovir alafenamide (TAF), and tenofovir (TFV). We evaluated concentrations of these antiretroviral (ARV) drugs in time-matched samples (maternal plasma, breast milk, and infant plasma) in a post hoc analysis of IMPAACT 2010, a randomized trial that evaluated three ARV treatment regimens in pregnancy.

**Methods:** Pregnant women with HIV in 9 countries were randomized 1:1:1 to start open-label ART with DTG + emtricitabine (FTC)/TDF, DTG + FTC/tenofovir disoproxil fumarate (TDF), or efavirenz (EFV)/FTC/TDF at 14-28 weeks of gestation. Matched maternal and infant samples were prospectively collected at random at Week 6 postpartum. Validated liquid chromatography-tandem mass spectrometry assays quantitated plasma and breastmilk concentrations of DTG, TAF, and TFV. The lower limit of quantitation for DTG, TAF, and TFV in breast milk and plasma was 7.8, 0.195, and 0.0077 ng/mL, respectively. Samples below the quantitation limit (BQL) were imputed to 0. The relative infant dose for each ARV was estimated by assuming an average milk intake of 150 mL/kg/day and each ARV’s observed breast milk concentrations.

**Results:** Data were available from 152 postpartum lactating women and their 192 predominantly breastfed infants. The average (SD) age of mothers at enrollment was 26 (6) years old, and most participants (85%) lived in Zimbabwe, Uganda, or Tanzania. Overall, 55% of infants were female with a mean (SD) gestational age of 40 (2) weeks, weight of 3089 (490) grams, and length of 50 (3) cm at birth. Median (range) maternal plasma concentrations of DTG, TAF, and TFV were 2810 (0.0-7460), 0.0 (0.0-158), and 96.1 (0.0-353) ng/mL, respectively. Breast milk (BM) and infant plasma (IP) concentrations are displayed in the Figure. The estimated median (range) relative infant dose of DTG, TAF, and TFV from breastfeeding was 1.92% (0.00-4.89), 0.00% (0.00-0.03), and 0.03% (0.00-0.11), respectively.

**Conclusion:** Breast milk transfer of DTG, TAF, and TFV is low and results in minimal systemic exposure in predominantly breastfed infants. The clinical relevance of subtherapeutic concentrations of these ARVs in breast milk is unknown but should be considered in the context of risk of drug resistance in infants who acquire HIV.
BM RNA VL (95% CI: 1.3-2.6), BM DNA VL was not detected in 26 (84%) cases and 59 (95%) controls. Only 3/14 (21%) case mothers on a TVF-containing regimen had detectable TFV levels in their plasma or BM vs 31/37 (84%) control mothers with detectable TFV in plasma and 29/37 (78%) in BM. In case mothers, plasma TFV levels were 10-fold lower (geometric mean ratio (95% CI): 0.11 (0.04-0.26) compared with controls, and BM TFV levels were 5-fold lower 0.18 (0.08-0.43) in case mothers compared with controls.

**Conclusion:** Odds of breastmilk HIV-1 transmission is associated with higher maternal plasma and BM VL and lower TFV levels.

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**927 Could This Be Cell-Associated Perinatal HIV Transmission?**

**Beatrice Cockbain**, Caroline Foster, Paula Seery, David Hawkins, Marta Boffito, Graham P. Taylor

**Background:** Recent French Perinatal Cohort data demonstrated no vertical transmissions among 5482 women living with HIV fulfilling the following criteria: effective ART from conception; undetectable plasma HIV RNA viral load (VL) near delivery; infant post-exposure prophylaxis (PEP); no breastfeeding. Despite fulfilling these criteria, we present two cases of vertical transmission from one UK centre and review the literature.

**Methods:** Retrospective case series of two infant HIV infections, without known vertical transmission risks. Review of maternal and infant notes. Literature review for cases of HIV transmission despite undetectable maternal plasma HIV RNA.

**Results:** Cases series. Both mothers conceived on triple ART. Excellent adherence reported. With an exception of 380 HIV RNA copies/ml (cpm) during pregnancy and around delivery (36 weeks GA for mother A; 36 weeks GA for mother B). Mother A had 6 weeks of enoxaparin from 11 weeks gestational age (GA) to 8 weeks in case A, both had multiple VL measures <50 cpm during pregnancy and around delivery (36 weeks GA for mother A; 36 weeks GA for mother B). Mother A had 6 weeks of enoxaparin from 11 weeks GA; mother B an iron infusion at 33 weeks GA. Both deliveries were term, one vaginal with induction of labour for pre-eclampsia at 38 weeks with <1 hour ruptured membranes, and one a planned pre-labour, pre-rupture of membranes Caesarean section at 39 weeks. Both infants had negative HIV VL at birth, had standard of care zidovudine PEP for 2–4 weeks as per contemporary guidelines and were exclusively formula-fed. HIV RNA was first detected in Infant A at 12 weeks (17.6 x 106 cpm) and in Infant B at 6 weeks (1.4 x 106 cpm). Literature review. One late transmission reported in Dolphin-2: Elavienz-based ART commenced week 28 GA with HIV VL undetectable at delivery, weeks 6, 12, 24, 48 and 72 post-partum. Exclusive breastfeeding to 24 weeks, breastmilk and solids to 48 weeks when breastfeeding stopped. The infant was HIV-negative on VL testing until week 72. HIV sequencing and phylogenetic analyses linked all infant and maternal viruses.

**Conclusion:** Without detectable maternal viremia, vertical HIV transmission is rare. Residual transmission may be from cell-associated virus, the dominant mode of transmission for other human retroviruses. ART may limit cell-associated HIV transmission. Exposure to maternal ART in utero may act as foetal pre-exposure prophylaxis and infant PEP protects for a limited period postnatally. Maternal lymphocyte persistence in the infant circulation, whether placental or gut-absorption, with cell-associated transmission, is likely implicated in these rare cases.
evaluated low (<50kg) and high (≥80kg) early pregnancy weight (before 24 weeks) and hypertension in pregnancy (SBP ≥ 140 or DBP ≥ 90 mm/Hg).

**Results:** Of 4,265 eligible individuals (median [IQR] age in pregnancy: 36 [32, 39] years), 1,102 (26%) switched from NVP to DTG and 3,163 (74%) did not switch from NVP prior to pregnancy. The most common backbones during pregnancy were TDF/3TC (90%), TDF/TDF (8%) for switchers and ZDV/3TC (60%) and TDF/TDF (39%) for non-switchers. Comparing switchers with non-switchers, RR (95% CI) values were 0.82 (0.75, 0.89) for any adverse and 0.84 (0.71, 1.00) for any severe adverse outcome. These differences were driven by SGA and very SGA (Figure, Left Panel). Switchers were less likely to have low and more likely to have high early pregnancy weight (Figure, Right Panel); the adjusted mean difference (95% CI) in early pregnancy weight was 2.9 (1.8, 4.1) kg. While switchers became pregnant in later years, sensitivity analyses indicated little evidence for time-trends in birth outcomes over the study period. Results were similar when excluding ZDV backbones.

**Conclusion:** Switching from legacy NVP-based regimens to DTG/TDF/TFC to pregnancy may reduce the risk of low maternal weight in early pregnancy and fetal growth restriction. This study provides further evidence that specific regimens impact birth outcomes, and that switching from legacy regimens prior to conception can improve birth outcomes.

**Figure.** Adverse birth outcomes (Left) and maternal pregnancy outcomes (Right), pre-conception switchers (NVP to DTG) versus non-switchers.

### 930 HIV and Syphilis Coinfection in Pregnancy and Adverse Birth Outcomes in Uganda

**Timothy Kintu**1, Mehal Churiwal1, Onemus Byamukama1, Ingrid V. Bassett1, Mark J. Siedner1, Anacret Byamukama1, Edna Timimwebwa1, Julian Adong1, Elias Kumbakumba1, Stephen Asiimwe1, Joseph Ngwazi1, Lisa M. Bebell2

1Mbarara University of Science and Technology, Mbarara, Uganda, 2Columbia University, New York, NY, USA

**Background:** The incidence of syphilis is increasing worldwide. Little is known about the combined impact of maternal HIV and syphilis coinfection on birth outcomes, especially in sub-Saharan Africa (sSA), where HIV prevalence is high.

**Methods:** We analyzed data from two prospective birth cohorts enrolled in southwestern Uganda from 2017 – 2023. All PHIV reporting taking antiretroviral therapy (ART). Participants were tested for syphilis using a Treponema pallidum particle agglutination (TP-PA) rapid test on peripheral blood (positive product term was not statistically significant.

**Conclusion:** Maternal HIV or TP-PA seropositivity did not increase the risk of adverse birth outcomes, though stillbirth incidence among TP-PA positive PHIV was higher than prior studies from sSA. High TP-PA seropositivity and RPR positivity among PHIV emphasize the need to improve prenatal care for PHIV with enhanced syphilis screening and treatment.

**Table. Predictors of adverse birth outcomes among pregnant women in Uganda.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unadjusted Odds Ratio</th>
<th>P-value</th>
<th>Adjusted Odds Ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal HIV infection</td>
<td>0.80 (0.60-1.14)</td>
<td>0.45</td>
<td>0.87 (0.47-1.59)</td>
<td>0.64</td>
</tr>
<tr>
<td>Maternal TP-PA seropositivity</td>
<td>1.06 (0.98 - 1.03)</td>
<td>0.06</td>
<td>1.65 (1.52 - 1.83)</td>
<td>0.00</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.98 (0.93 - 1.03)</td>
<td>0.01</td>
<td>0.86 (0.93 - 1.00)</td>
<td>0.51</td>
</tr>
<tr>
<td>Hypertensive disorder during pregnancy</td>
<td>0.76 (0.50 - 1.14)</td>
<td>0.16</td>
<td>0.68 (0.44 - 1.04)</td>
<td>0.08</td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td>0.75 (0.66 - 0.84)</td>
<td>0.01</td>
<td>0.91 (0.86 - 0.97)</td>
<td>0.005</td>
</tr>
</tbody>
</table>


**Benjamin H. Chi**1, Deborah Kacakane1, Emily Brown1, K. Rivet Amico2, Sharon Huang1, Teacer Nemadazira1, Elizea Horne1, Clemensia Nakabito1, Sharon K. Mambiya1, Violet Korutaro1, Benjamin Johnston3, James F. Rooney1, Lynda Strandis-Chibanda1, for the IMPAACT 2009 Study Team

1University of North Carolina at Chapel Hill, Chapel Hill, NC, US; 2Harvard TH Chan School of Public Health, Boston, MA, USA; 3University of Zimbabwe, Harare, Zimbabwe

**Background:** FTC-TDF-based PrEP is recommended for pregnant people in settings of high HIV transmission. However, few have evaluated uptake, maternal safety, and pregnancy outcomes in the context of a clinical trial.

**Methods:** The PrEP Comparison Component of IMPAACT 2009 enrolled pregnant participants aged 16–24 years at <32 weeks gestation in Malawi, South Africa, Uganda, and Zimbabwe. Participants were enrolled in parallel cohorts based on choice to initiate or decline daily oral FTC-TDF for PrEP at entry. All were followed in pregnancy to 6 months postpartum and could start or stop PrEP at any time. While on PrEP, they received Integrated Next Step Counseling, regular drug level feedback via TFV-DP from dried blood spots, and weekly two-way text messaging. Adverse events (AEs) were graded per DAIDS toxicity standards.

**Results:** From March to December 2022, 350 eligible participants enrolled (mean age: 21 years; median gestational age: 24 weeks). Among 335 participants with pregnancy outcome information, 233 initiated PrEP in pregnancy (229 at enrollment, 4 later). Another 117 declined at entry and never initiated PrEP during pregnancy. Median duration of antenatal PrEP use was 11 weeks (IQR: 7–15). A total of 31 (9%) participants experienced at least one grade ≥3 AE through delivery, with a greater proportion among PrEP initiators (11.2%, 95% CI: 7.4–15.9%) vs. decliners (4.3%, 95% CI: 1.9–9.7%). Most frequent grade ≥3 AEs were complications of pregnancy or delivery (9% for initiators vs. 3% for decliners). None were considered related to PrEP use.

**Conclusion:** Our findings further support the safety of FTC-TDF in pregnancy. Despite the high occurrence of AEs, none appeared related to PrEP use. While other PrEP modalities undergo evaluation for antenatal populations, daily oral FTC-TDF remains a safe and essential component of HIV prevention in pregnancy.
Outcomes Following Prenatal Exposure to Raltegravir: A Multi-Cohort European Study

Rebecca Sconza1, Georgina Fernandes1, Karoline Aebi-Popp1, Luminenta Ene1, Antoninette Frick1, Anna Gamelli2, Marta Illán Ramos3, Christian Kahler3, Helen Peters4, Luis M. Prieto Tato5, Anna Samurina6, Carla Giaquinto6, Claire Thorne6, for the EPPICPC Pregnancy Study Group

1UO 2 Great Ormond Street Institute of Child Health, London, United Kingdom, 2University Hospital of Bern, Bern, Switzerland, 3Dr Victor Babey Timanco Infecious Diseases and Paanepneumontology Clinical Hospital, Bucharest, Romania, 4Hospital Universitario de la Vall d’Hebron, Barcelona, Spain, 5Hospital Sant Joan de Deu Barcelona, Barcelona, Spain, 6Hospital Universitario Clínico San Carlos, Madrid, Spain, 7Children’s Hospital of Eastern Switzerland St. Gallen, St Gallen, Switzerland, 8Hospital Universitàrio do Norte de October, Madrid, Spain, 9St. Petersburgh Center for Prevention and Control of AIDS and Infectious Diseases, St Petersburg, Russian Federation, 10University of Padova, Padova, Italy

Background: Real world data on safety of antiretroviral drugs (ARVs) in pregnancy informs decision-making, but accumulating sufficiently large samples with specific periconception ARV exposure to rule out increased risk of rare birth defects, such as neural tube defects (NTDs), can take many years.

Methods: We assessed risk of birth defects and other adverse outcomes following prenatal exposure to raltegravir (RAL) using pooled prospectively collected individual patient data from studies in the European Pregnancy and Paediatric Infections Cohort Collaboration (EPPICC). Pregnancies with any documented prenatal exposure to RAL and with outcomes in 2008-2020 were included. Earliest prenatal RAL exposure timing was classified as periconception (PC) (exposure at ≤6 completed gestational weeks [GWs]), first trimester (T1) (exposure in T1 at >6 completed GWs), and second/third trimester (T2/T3) (exposure at >6 completed GWs).

Results: A total of 1499 pregnancies across 9 cohorts were included (1194, 79.7% from the UK). Most pregnancies were in women of Black (898/1480, 60.7%) or white (466/1480, 31.5%) ethnicity. Median age at conception was 32 years (IQR: 27-36). Half (763/1487, 51.3%) of RAL-exposed pregnancies were conceived on ARVs. There were 1429 live births (1466 live-born infants), 10 with preterm birth (<37 weeks’ gestation), 13 with NTDs, 5 with severe congenital anomalies (SCAs) and 6 with non-SCAs (other). One NTD was observed (spina bifida with PC exposure).

Conclusion: The birth defect rate in EPPICC is consistent with and contributes to the current evidence base on periconception RAL use.

Table. Birth outcomes of infants exposed to ≥7 days of Raltegravir during Gestation

Table. Birth outcomes of infants exposed to ≥7 days of Raltegravir during Gestation

934 First Trimester Exposure to Newer Antiretroviral Agents and Congenital Anomalies in a US Cohort

Kelly Fung1, Sonia Hernandez-Diaz2, Rebecca Zash3, Ellen G. Chadwick4, Russell Van Dyke4, Carly Broadwell4, Jennifer Jao5, Kathleen M. Powis1, Lynn Yee6, Paige L. Williams7, for the Pediatric HIV/AIDS Cohort Study (PHACS)

1Harvard TH Chan School of Public Health, Boston, MA, USA, 2Beth Israel Deaconess Medical Center, Boston, MA, USA, 3Northwestern University, Chicago, IL, USA, 4Tufts University, Medford, MA, USA, 5University of Nebraska Medical Center, Omaha, NE, USA, 6Baylor College of Medicine, Houston, TX, USA

Background: The teratogenicity of antiretroviral medications (ARVs) is a key consideration in clinical recommendations and prescribing decisions for HIV regimes used during pregnancy. However, in the U.S., data on the association between ARV exposures and major congenital anomalies are generally limited to older ARV agents, many of which are now rarely used. Given the timing of organogenesis, it is critical to assess the safety of first-trimester fetal exposure to newer ARVs.

Table. Birth outcomes of infants exposed to ≥7 days of Bictegravir during Gestation

Table. Birth outcomes of infants exposed to ≥7 days of Bictegravir during Gestation

932 Outcomes Following Prenatal Exposure to Raltegravir: A Multi-Cohort European Study

Rebecca Sconza1, Georgina Fernandes1, Karoline Aebi-Popp1, Luminenta Ene1, Antoninette Frick1, Anna Gamelli2, Marta Illán Ramos3, Christian Kahler3, Helen Peters4, Luis M. Prieto Tato5, Anna Samurina6, Carla Giaquinto6, Claire Thorne6, for the EPPICPC Pregnancy Study Group

1UO 2 Great Ormond Street Institute of Child Health, London, United Kingdom, 2University Hospital of Bern, Bern, Switzerland, 3Dr Victor Babey Timanco Infecious Diseases and Paanepneumontology Clinical Hospital, Bucharest, Romania, 4Hospital Universitario de la Vall d’Hebron, Barcelona, Spain, 5Hospital Sant Joan de Deu Barcelona, Barcelona, Spain, 6Hospital Universitario Clínico San Carlos, Madrid, Spain, 7Children’s Hospital of Eastern Switzerland St. Gallen, St Gallen, Switzerland, 8Hospital Universitàrio do Norte de October, Madrid, Spain, 9St. Petersburgh Center for Prevention and Control of AIDS and Infectious Diseases, St Petersburg, Russian Federation, 10University of Padova, Padova, Italy

Background: Real world data on safety of antiretroviral drugs (ARVs) in pregnancy informs decision-making, but accumulating sufficiently large samples with specific periconception ARV exposure to rule out increased risk of rare birth defects, such as neural tube defects (NTDs), can take many years.

Methods: We assessed risk of birth defects and other adverse outcomes following prenatal exposure to raltegravir (RAL) using pooled prospectively collected individual patient data from studies in the European Pregnancy and Paediatric Infections Cohort Collaboration (EPPICC). Pregnancies with any documented prenatal exposure to RAL and with outcomes in 2008-2020 were included. Earliest prenatal RAL exposure timing was classified as periconception (PC) (exposure at ≤6 completed gestational weeks [GWs]), first trimester (T1) (exposure in T1 at >6 completed GWs), and second/third trimester (T2/T3) (exposure at >6 completed GWs).

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Conclusion: The birth defect rate in EPPICC is consistent with and contributes to the current evidence base on periconception RAL use.

Table. Birth outcomes of infants exposed to ≥7 days of Bictegravir during Gestation

Table. Birth outcomes of infants exposed to ≥7 days of Bictegravir during Gestation

933 Birth Outcomes Following Bictegravir use During Pregnancy

Rosemary M. Olivero1, Paige L. Williams7, George Sawyer4, Lynn Yee6, Kunjal Patel6, Sonia Hernandez-Diaz2, Kathleen M. Powis1, Mary Paul5, Ellen G. Chadwick4, for the Pediatric HIV/AIDS Cohort Study (PHACS)

1Carnell Health Helen DeVos Children’s Hospital, Grand Rapids, MI, USA, 2Harvard University, Boston, MA, USA, 3Northwestern University, Chicago, IL, USA, 4Harvard University, Cambridge, MA, USA, 5Boston College of Medicine, Houston, TX, USA

Background: Bictegravir (BIC), co-formulated in a tablet with tenofovir alafenamide and emtricitabine, is being increasingly prescribed to pregnant persons with HIV (PWH), yet limited birth outcome data have been reported.

Methods: We conducted a descriptive analysis of PWH 18-45 years of age enrolled in at least one Pediatric HIV/AIDS Cohort Study (PHACS)-affiliated protocol who received BIC for at least 7 days during pregnancy and completed follow-up through delivery. The outcomes of interest were gestational age at birth, preterm birth (<37 weeks’ gestation), gestational-age adjusted birth weight (BWZ) and length (BLZ) z-scores (CDC 2000 growth standards), small for gestational age (SGA, birthweight <10th percentile), congenital anomalies, neonatal deaths in the first 28 days of life, and infant HIV status. The prevalence (with Clopper-Pearson 95% Confidence Intervals [CI]) of each outcome was calculated among infants exposed to BIC during gestation and, for the outcome of anomalies, in infants exposed during the first trimester. Maternal CD4 count and HIV viral load (VL) nearest and prior to delivery were reported if available.

Results: A total of 144 infants were born to 134 unique PWH (including 2 twin sets) who received BIC for at least 7 days during pregnancy. Infants were born between September 2018 and October 2023. Median maternal age at delivery was 29.7 years (interquartile range [IQR]: 26.1, 33.9), 71% reported their race/ethnicity as Black or African American, and 52% had an annual household income ≤$20,000; all US census regions were represented. Fifty-three percent initiated BIC prior to conception. The mean gestational age was 38.2 weeks [standard deviation (SD)=1.5), the prevalence of preterm birth was 14.0% (95% CI: 8.8%, 20.8%) and SGA was 10.6% (95% CI 6.0%, 16.8%). Mean BWZ and BLZ were -0.49 (SD 0.93) and 0.08 (SD 1.13), respectively (Table). No neonatal deaths or perinatal HIV transmissions were reported. Among 99 infants exposed to BIC in the first trimester, 5 (5.1%, 95% CI 1.7%, 11.4%) had at least one congenital anomaly reported (Table). Maternal V/L was <50 copies/mL in 82.0% and median CD4 count was 466.5 (IQR: 309.0, 744.0) cells/mm3 nearest to delivery of exposed infants.

Conclusion: In this US cohort, BIC use during pregnancy was frequent. These findings provide initial reassuring observations about the potential safety of BIC use during pregnancy, although comparative data and continued surveillance of outcomes of antiretroviral therapy among PWH is warranted.
Methods: We evaluated the association between first trimester exposure to newer ARVs and major congenital anomalies among infants born between 2012-2022 to pregnant women with HIV enrolled in the U.S.-based prospective Surveillance Monitoring for ART Toxicities (SMARTTY) study conducted by the PHACS network. First trimester exposures to newer ARVs were abstracted from maternal medical records. Trained study site staff conducted physical exams and abstracted congenital anomalies from infant medical records, and investigators classified the anomalies using the Metropolitan Atlanta Congenital Defects Program classification system. The prevalence of major congenital anomalies identified by age 1 year was estimated for infants exposed and unexposed to each ARV. Generalized estimating equation (GEE) models were used to estimate the odds ratio (OR) and confidence interval (CI) of major congenital anomalies for each ARV exposure compared to those unexposed to that ARV, adjusting for infant birth year, maternal age at delivery, pre-pregnancy BMI, pregestational diabetes, and first trimester alcohol use, and accounting for correlation among siblings and/or multifetal births.

Results: Of 2034 eligible infants, major congenital anomalies occurred in 135 (6.6%; 95% CI: 5.6%-7.8%). Cardiovascular (n=43) and musculoskeletal (n=37) anomalies were most common. The adjusted odds ratios (95% CI) of congenital anomalies were 1.07 (0.64-1.78) for darunavir, 0.93 (0.47-1.85) for raltegravir, 1.04 (0.58-1.85) for rilpivirine, 1.25 (0.67-2.33) for elvitegravir, 0.75 (0.36-1.57) for dolutegravir, and 0.34 (0.05-2.33) for bictegravir (see Table). Findings were similar after adjustment for nongenital side effects in the ARV regimen.

Conclusion: The odds of congenital anomalies among infants with first trimester exposure to newer ARVs did not differ substantially from that among infants unexposed to these specific ARVs. However, modest effects cannot be ruled out, highlighting the need for continued evaluation of these associations in larger populations.

935 Cancer Incidence in Children Who Are HIV-Exposed and Uninfected in England: Data Linkage Study
Lauretta L. Bukasa, Claire Thorne, Mario Cortina-Borja, Helen Peters, Pia Hardell
University College London, London, United Kingdom

Background: Children born to women living with HIV develop in an in utero environment that includes exposure to HIV and antiretroviral therapy (ART). However, the long-term health implications of these exposures for children who are HIV-exposed and uninfected (CHEU) are largely unknown.

Methods: Population-based surveillance data from children born to women living with HIV in England (ISOSIS) between 1995 and 2022 were linked to national cancer registration and mortality data. Date of last cancer registration was used where >1 cancer event occurred. Age- and sex-standardised incidence ratios (SIR) were used to compare cancer incidence in CHEU with the general child population in England. Cancer incidence and associations with maternal, HIV/ART and child characteristics were estimated using Cox-proportional hazards models.

Results: There were 19 cancer events (13 in females, 6 in males) in 17 children among 14047 CHEU records with 159,241 person-years follow-up. The cancer incidence rate was 1.07 (95% CI: 0.62-1.71). All cancer events occurred before 17 years of age and median age at last cancer event was 4 years (IQR: 7, Q12–Q3:9). Cancers affecting the central nervous system were most common (n=6), followed by lymphoid, haematopoietic, and related tissue cancers (n=4).
937 Brain Structure of South African HEU Children Exposed to Dolutegravir Versus Efavirenz

Layla E. Bradford1, Jessica E. Ringshaw1, Catherine J. Wedderburn1, Niall J. Bourke1, Helene Theunsissen1, Thokozile R. Malaba1, Lauren Davet1, Nengjie He1, Helen Reynolds5, Angela Colbers4, Duolao Wang4, Saye Khoo4, Landon Myer1, Kirsten A. Donald1
1University of Cape Town, Cape Town, South Africa, 2King’s College London, London, United Kingdom, 3Boston Children’s Hospital, Boston, MA, USA, 4Radboud University Medical Center, Nijmegen, Netherlands, 5University of Liverpool, School of Tropical Medicine, Liverpool, United Kingdom

Background: Current research suggests that children who are HIV-exposed and uninfected (CHEU) may be at risk for neurodevelopmental delay and altered structural brain development compared to children who are HIV-unexposed (CHU), however the specific factors driving this association and the potential role of specific antiretroviral regimens are poorly understood. In particular volumes of specific regions of the brain may be reduced in CHEU compared to CHU, however, there is no research published on the structural brain outcomes of children born to mothers who received DTG- or EFV-based ART.

Methods: We collected high resolution magnetic resonance (T1-weighted) scans from the DOLPHIN-2 Plus, an open-label follow-up to the DOLPHIN-2 trial (NCT03249181). In this analysis, total grey matter and total subcortical volumes were compared between CHEU and CHU groups using multivariate analysis of variance (MANOVA) adjusting for age, sex and total intracranial volume. Within the CHEU group, brain volumes were compared between children who were born to mothers who received DTG and EFV-based ART.

Results: Between 2021 and 2023, 24 CHEU (12 DTG, 12 EFV; mean age 45 months; 54% male) born in the DOLPHIN-2 trial were enrolled and scanned at 2-4 years along with 64 CHU (mean age 43 months; 56% male). Demographic characteristics were similar for both CHEU vs CHU groups. In unadjusted and adjusted analyses, there were no differences for an effect of HIV exposure on total grey matter (adjusted p=0.642) or subcortical grey matter (p=0.549). Similarly, ART exposure showed no significant association with total grey matter (p=0.869), or subcortical grey matter (p=0.097), although there was a trend towards smaller subcortical volumes in the EFV compared to DTG groups (F=3.05, partial Eta squared =0.138).

Conclusion: Total grey matter brain volumes were similar in CHEU and CHU at 3-4 years of age in this sample. These are the first data comparing brain volumes in HEU children born to mothers receiving DTG- versus EFV-based ART in pregnancy. While there were no significant differences by ART regimen, the trend for larger subcortical volumes in DTG-exposed children in this small sample requires further investigation.

938 Neurodevelopment in Children Exposed In Utero to Dolutegravir- or Efavirenz-Based ART in Botswana

Adam R. Cassidy2, Gloria K. Mayondo1, Kehabibe Moabi1, Allison LeMahieu1, Paige L. Williams1, Naledi Kamanga2, Kathleen M. Powis1, Dinah Ramaabya1, Francis Banda1, Joseph M. Makhema2, Betsy Kammerer1, Shahn Lockman2
1Mayo Clinic, Rochester, MN, USA, 2Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

Background: Dolutegravir (DTG)- and, to a lesser extent, efavirenz (EFV)-based antiretroviral treatment (ART) regimens are commonly used by pregnant women living with HIV in high burden HIV settings, increasingly with initiation prior to conception. Little is known about the impact of in utero exposure to DTG- or EFV-based ART on child neurodevelopmental (ND) outcomes, although some prior data have raised concerns for EFV exposure.

Methods: We prospectively enrolled 3 cohorts of 2-year-old children: HIV-exposed/uninfected (HEU)/DTG-exposed, HEU/EVF-exposed, and HIV-unexposed/uninfected (HUU), from March 2021-May 2023. DTG or EFV were taken in combination with TDF/FTC or TBC. Child ND status was assessed using the Bayley-III (BSID-III), BRIEF-P-3F, and CREDI (Short; Social-Emotional). ND outcomes were compared between DTG- and EFV-exposed groups, and between HEU and HUU groups, using GEE models to account for twins, and adjusting-for enrollment site, child sex/age at testing, maternal age/education/marital status/income, food insecurity (and for DTG vs EFV comparisons, breastfeeding and timing of first in utero ART exposure). Children were classified “at risk” if they scored ≥1SD below the mean or were unable to complete the BSID-III.

Results: A total of 564 children (202 HEU/EVF, 202 HEU/DTG, 160 HUU; Mage=25.7 months; 49% female) participated. Means were similar across ART-exposure groups in unadjusted and adjusted models (Figure). Adjusted relative risk (aRR) of “at risk” classification was lower in children who were DTG-exposed than EFV-exposed on BSID-III Cognitive (4.5% vs. 8.4% aRR=0.36 (95%CI0.10, 0.78)) and Expressive Language (10.9% vs 17.3%, 0.61 (0.38, 0.98)) domains. Children HEU (EFV+DTG) were more likely than children HUU to be classified “at risk” on BSID-III Expressive Language (14.1% vs 7.5%, aRR=1.89 (1.00, 3.60)). Children HEU were rated as having slightly better executive function skills on the BRIEF-P-3F. Inferences were similar in sensitivity analyses controlling for perinatal birth status.

Conclusion: Two-year ND outcomes among HEU and HUU children in Botswana were mostly comparable. However, consistent with prior studies, HEU status was associated with higher risk of adverse language outcomes. Among children HEU, those exposed in utero to EFV-ART were at higher risk of adverse cognitive and expressive language outcomes than DTG-ART-exposed. Longer-term ND follow-up is needed to examine the possibility of increased ND burden as HEU-/ART-exposed children enter school-age.

939 Prenatal PrEP Exposure and Neurodevelopment Among Children at 48 Months

Lauren A. Gomez1, John Kinuthia1, Felix Abuna1, Sarah Benki-Nugent2, Julia Dettinger3, Anna Larsen1, Mary Marwa1, Ben Ochieng1, Nancy Ngumbao1, Salphine Watoy1, Joshua Sterni1, Barbra Richardson1, Grace John-Stewart1, Jillian Pintye3
1University of Washington, Seattle, WA, USA, 2Kenyatta National Hospital, Nairobi, Kenya, 3King's College London, London, UK

Background: Data on neurodevelopmental outcomes following in utero ART exposure remains scarce and most studies to date are among women living with HIV and their infants who have both ART and HIV exposure. We assessed the relationship between prenatal PrEP exposure and child neurodevelopment through 48 months among mother-child pairs without HIV.

Methods: Data from women enrolled in a cluster RCT (NCT03070600) evaluating PrEP delivery strategies at 20 antenatal clinics in Western Kenya were analyzed. HIV-negative women were enrolled and offered oral tenofovir disoproxil fumarate (TDF)-based PrEP during pregnancy and followed through 9 months postpartum regardless of PrEP status. A subset were enrolled into an extension cohort at 4 sites to be followed until their children reached 5 years. Neurodevelopment was assessed by trained nurses at 36 and 48 months using the Malawi Developmental Assessment Tool (MDAT), a validated instrument that evaluates social, language, fine motor, and gross motor domains. The association between prenatal PrEP exposure and MDAT domain scores at 36-48 months was evaluated using linear regression models clustered by facility and adjusted for gestational age at birth, maternal age and education, infant sex, and partner HIV status.

Results: As of September 2023, 648 mother-child pairs had children aged 36-48 months and were included in the analysis, 21% had any PrEP exposure for a median duration of 3.3 months (IQR: 2.4-4.3) during pregnancy. Compared to mothers who did not take PrEP, mothers who took PrEP in pregnancy were more likely to have only primary school education (70% vs. 56%; p=0.004) and to have a partner known to be living with HIV of unknown status (67% vs 37%; p<0.001). There was no difference in mean MDAT score for any domain at 36 months (adjusted mean differences: social 0.07, 95% CI: -1.61, 1.76, p=0.92; language 0.54, 95% CI: -1.57, 2.66, p=0.58; fine motor -0.50, 95% CI: -1.49, 0.49, p=0.29; gross motor -0.17, 95% CI: -1.19, 0.85, p=0.72). Results were similar at 48-months with no differences between exposure groups.

Conclusion: No differences in neurodevelopment from 36-48 months were observed between children with and without prenatal PrEP exposure. Our results support a growing body of evidence demonstrating the safety of prenatal PrEP use and add to the limited data on neurodevelopmental outcomes in PrEP exposed children.
940 Simplifying Dosing by Harmonizing Weight-Band-Based Dosing Across Therapeutic Areas in Children

Hylke Waalwijn1, Mouner Almeit1, Roeland E. Wasmann1, Tim R. Cressey1, Philippa Easterbrook2, Peter Ehizibue Olumese3, Annette C. Hesseling4, Joel Tarning5, Anna Turkova6, Elin Svensson7, Angela Colbers8, Wilson M. Were9, Paolo Denti10, Martina Penazzato11

1University of Cape Town, Cape Town, South Africa, 2Chiang Mai University, Chiang Mai, Thailand, 3World Health Organization, Geneva, Switzerland, 4Desmond Tuto TB Centre, Western Cape, South Africa, 5Mahidol University, Bangkok, Thailand, 6University College London, London, United Kingdom, 7Uppsala University, Uppsala, Sweden, 8Radboud University Medical Center, Nijmegen, Netherlands

Background: Pediatric WHO dosing guidance recommends using dosing weight bands, but these are not standardized across therapeutic areas. This adds to the complexity of drug prescribing and administration when treating individual children for multiple diseases and comorbidities, increasing the risk of dosing errors. Harmonized weight bands across therapeutic areas are expected to simplify dosing, relieve the burden on practitioners and caregivers, reduce dosing errors, and provide clear dosing guidance for future pediatric drug development programs. However, this cannot come at the expense of drug efficacy or safety. We investigated the impact of harmonizing weight bands for HIV, tuberculosis (TB), malaria, and Hepatitis C (HCV) treatment, based on simulated drug exposures, with the goal of providing evidence for future harmonization of pediatric weight bands.

Methods: Weight bands recommended in the Pocket Book for Hospital Care in Children, WHO Antibiotic Book, and guidance documents for HIV, TB, malaria, and HCV were selected to assess the potential for harmonization. Using available population pharmacokinetic models for individual drugs, the impact of harmonizing weight bands on drug exposures were simulated and the corresponding changes discussed with panels of clinical and pharmacological experts.

Results: The proposed weight bands align with those recommended in the WHO antibiotic book and the pocket book. Table 1. For drugs used in HIV, HCV, and for treatment and prevention of drug-susceptible TB, harmonization had minimal impact on drug exposures and are not expected to affect drug efficacy or safety. For children weighing <10 kg treated for drug-resistant TB, harmonization had a pronounced impact; nevertheless, safe, and effective exposures are expected after optimizing drug doses based on age to account for maturation of elimination pathways. Safe and effective exposures are expected with harmonized dosing for 7/9 priority malaria drugs and for all 3 drugs currently used for seasonal malaria chemoprevention.

Conclusion: This work demonstrates that harmonized pediatric weight bands, with additional age-based dosing for infants, provides sufficient flexibility for safe and effective pediatric dosing for treatment of HIV, HCV, TB, and seasonal malaria chemoprevention. For malaria treatment, additional safety, and efficacy studies are recommended due to expected exposures in 2/9 priority drugs. This analysis is expected to inform future revision of WHO guidelines.

Table 1. Differences between the proposed harmonized weight bands and weight bands currently recommended in WHO dosing guidance for general hospital care, antibiotics, HIV, TB, malaria, and HCV: red weight bands affected by harmonization. DT-B: drug-susceptible tuberculosis; HLV-MDR-TB: rifampin-resistant/multi-drug-resistant tuberculosis.

<table>
<thead>
<tr>
<th>Harmonized weight bands (kg)</th>
<th>3</th>
<th>5.5</th>
<th>6</th>
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<th>14.9</th>
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<td>DT-B</td>
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<td>HCV</td>
<td>1/4 prefered treatment combinations aligned with harmonized weight bands</td>
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941 Single-Dose PK/Safety of DTG-Dispersible Tablets in Neonates Supports Multi-Dosing: PETITE-DTG Study

Adrie Bekker1, Nicolas Salvadori2, Helena Rabie1, Samantha du Toit3, Kanchana Than-in-att3, Maria Groenewald4, Andrew Owen1, Rachitda Cressay1, Marc Lallemant5, Mark F. Cotton6, Tim R. Cressay7, for the PETITE-DTG Study Team

1Stellenbosch University, Cape Town, South Africa, 2Chiang Mai University, Chiang Mai, Thailand, 3University of California San Diego, San Diego, CA, USA, 4University of Liverpool, Liverpool, United Kingdom, 5AMS-PHPT Research Collaboration, Chiang Mai University, Chiang Mai, Thailand

Background: Dolutegravir dispersible tablets (DTG-DT) are approved for infants ≥3 kg and aged >4 weeks but their suitability for neonates remains unknown. We evaluated the pharmacokinetics (PK) and safety of pediatric DTG-DT in neonates born to women on DTG-based therapy.

Methods: PETITE-DTG is an ongoing phase I/II, open-label, single center, two-stage trial in South Africa evaluating the PK and safety of DTG-DT (10 mg, scored) in term neonates (birth weights ≥2kg). Stage 1 was designed to assess single doses of DTG on top of standard ARV prophylaxis to inform a multi-dose strategy in Stage 2. In Stage 1, a single 5 mg DTG-DT dose was administered to 8 neonates between ≥14 & <28 days of life (Cohort 1A) followed by intensive PK and safety assessments. After no safety signal was observed, 8 additional neonates received a single 5 mg DTG-DT dose at <14 days of life (Cohort 1B) followed by identical PK and safety assessments. A population PK model was developed using Stage 1 data, and different dosing scenarios were simulated to select the optimal 5 mg DTG multi-dose strategy through 28 days of life (target DTG criteria: geometric mean GM C<sub>min</sub> >0.67 µg/mL and individual C<sub>min</sub> ≥17.0 µg/mL).

Results: 16 neonates, 8 per Cohort, completed Stage 1. Median (range) birth weight was 3.1 (2.6-4.2) kg and PK sampling performed between 3 to 22 days of life (Figure a). Single dose DTG C<sub>min</sub> ranged from 2.0-6.6 µg/mL. No grade ≥3 adverse events (AEs) were reported, and no AEs were related to DTG. One neonate required hospitalization for a skin rash (grade 2). A one-compartment PK model described DTG plasma concentrations, with clearance (CL/F) influenced by weight and postnatal age. DTG CL/F rapidly increased after birth with >6-fold change between day 1 and 14 of life. Simulations predicted >10% exceeded GM C<sub>min</sub> >0.67 µg/mL and individual C<sub>min</sub> >17.0 µg/mL.

Results: 16 neonates, 8 per Cohort, completed Stage 1. Median (range) birth weight was 3.1 (2.6-4.2) kg and PK sampling performed between 3 to 22 days of life (Figure a). Single dose DTG C<sub>min</sub> ranged from 2.0-6.6 µg/mL. No grade ≥3 adverse events (AEs) were reported, and no AEs were related to DTG. One neonate required hospitalization for a skin rash (grade 2). A one-compartment PK model described DTG plasma concentrations, with clearance (CL/F) influenced by weight and postnatal age. DTG CL/F rapidly increased after birth with >6-fold change between day 1 and 14 of life. Simulations predicted >10% exceeded GM C<sub>min</sub> >0.67 µg/mL and individual C<sub>min</sub> >17.0 µg/mL.

Conclusion: Single doses of 5 mg DTG-DT were safe in neonates. Due to slow DTG CL/F in early life, 5 mg DTG-DT was not optimal from birth. In Stage 2, the safety and PK of 5 mg DTG-DT for the first 2 weeks of life, followed by q24 through 28 days of age, will be assessed. Both the DTG-DT and the recently approved 5 mg DTG oral dispersible film will be investigated.
High Drug Exposures in Neonates Using ABC/3TC Dispersible Tablets During the First Week of Life

Navarat Panjasawatwong, Adrie Bekker, Helena Rabie, Nicolas Salvadori, Samantha du Toit, Ratchada Cressey, Marcel Lillamant, Mark F. Cotton, Tim R. Cressey, for the PETITE Study Team

Background: Pediatric fixed dose combination (FDC) formulations are preferred for infants and young children but rapid maturation of metabolic and elimination pathways complicate their use in neonates. We previously reported the pharmacokinetics (PK) and safety of abacavir/lamivudine (ABC/3TC) FDC dispersible tablet in neonates but no PK data were available prior to 6 days of life. Our objective was to develop population PK models to estimate ABC/3TC exposures in neonates using ABC/3TC dispersible tablets from birth.

Methods: The ‘PETITE’ study was a phase II, open-label, single-arm, two-stage pharmacokinetic and safety trial. HIV-exposed neonates received 30/15 mg of ABC/3TC (¼ dispersible tablet) once daily and 80/20 mg of LPV/r (2 sachets) twice daily through 28 days of life. Each neonate had intensive PK sampling on two occasions between 6 and 24 days of life. Safety visits were performed 1-2 weeks after each PK visit. PK models of ABC and 3TC were developed using nonlinear mixed-effects modeling. Monte Carlo simulations (n=5,600 virtual neonates) were performed to predict ABC and 3TC exposures from birth through 28 days of life. Geometric mean (GM) ABC and 3TC target exposures (AUCO-24) reported in children were 6.3 to 50.4 and 6.3 to 26.5 mg.hr/L for ABC and 3TC, respectively.

Results: 16 term neonates (8 females) with a median birth weight of 3,140 g were included. One compartment PK models with first-order absorption and elimination best described both ABC and 3TC plasma concentrations, incorporating body weight and postnatal age on clearance as surrogates of hepatic and renal maturation. Simulations predicted that maximum ABC and 3TC exposures occurred at 2 days of life. The predicted GM ABC AUCO-24 decreased by 28% between 2 and 7 days of life and remained above the target during the first week of life (Figure 1(a)). The GM 3TC AUCO-24 decreased by 26% between 2 and 4 days of life and remained above the target during the first 4 days of life (Figure 1(b)).

Conclusion: Administering 30/15 mg of ABC/3TC (¼ dispersible tablet) once daily to neonates from birth leads to plasma exposures during the first week of life above those observed in young children. While no safety issues were reported in the PETITE study, continued safety surveillance of the ABC/3TC dispersible tablets in neonates is warranted, particularly when started in the first week of life.

Figure: Simulated Geometric Mean (GM) (a) ABC and (b) 3TC AUCO-24 versus postnatal age. Shaded areas represent the GM target ranges. 6.3-50.4 and 6.3 to 26.5 mg.hr/L for ABC and 3TC, respectively.

A Single Once Daily ABC/DTG/3TC Tablet Predicts Safe and Effective Exposures in Children 3 to <6kg

Hardik Chandrasana, Kristina M. Brook, Ann M. Buchanan, Lionel Tan, Hilda A. Mujuru, Angela Colbers, Judy Hopking, Manika Cuffia, Michael McKenna, Andrew Wiznia, Sean Brummel, Adrie Bekker, Tim R. Cressey, Helena Rabie

Background: Abacavir (ABC)/dolutegravir (DTG)/lamivudine (3TC) is a fixed-dose combination (FDC) tablet approved for adults and children with HIV weighing ≥6kg and aged ≥3 months. We evaluated whether ABC/DTG/3TC...
Population Pharmacokinetics of ABC/DTG/3TC FDC to Support Dosing in Infants with Early HIV Diagnosis

**Background:** Once-daily fixed-dose combinations (FDC) containing abacavir (ABC), dolutegravir (DTG), and lamivudine (3TC) have been approved for use in the US for children with HIV weighing ≥6 kg. The once-daily single tablet treatment option may be a practical solution for infants with early HIV diagnosis.

**Methods:** ABC, DTG, and 3TC pediatric population pharmacokinetic (PopPK) models using multiple PopPK parameters. Exposures were then simulated across weight bands for each drug and compared with pre-defined exposure target ranges (DTG C24 geometric mean [GM] 0.697-2.26 μg/mL, ABC AUC0-24 GM 6.3-50.4 μg*h/mL, and 3TC AUC0-24 GM 6.3-26.5 μg*h/mL). We reviewed safety findings for ABC, DTG and 3TC in the lowest weight bands (WBs) of three pediatric trials (P1093, ODYSSEY and IMPAACT 2019), alongside available literature describing PK and safety of ABC and 3TC in neonates and infants under 3 months (including PETITE Study).

**Results:** Predicted GM steady-state plasma exposures of ABC, DTG and 3TC in children 3-<6kg receiving a single FDC of ABC/DTG/3TC DT (Table 1) were within the target ranges for both component. AUC(0-24h) C24h, and C24h of ABC, DTG and 3TC were also comparable to prior pediatric and adult studies. Review of pediatric safety data showed similar safety profiles across WBs and were consistent with the known safety profile of the individual drugs. Most children in these studies were on the higher WHO doses of 3TC 60mg and ABC 120mg for this WB. Conclusion: Predicted drug exposures support the potential use of a single FDC of ABC/DTG/3TC DT in infants weighing 3-<6kg (aged ≥4 weeks), with efficacy and safety expected to be comparable to prior pediatric studies in children ≥6kg. The once daily single tablet treatment option may be a practical solution for infants with early HIV diagnosis.

**Table 1. Predicted ABC/DTG/3TC Exposures**

<table>
<thead>
<tr>
<th>Weight Band</th>
<th>ABC</th>
<th>DTG</th>
<th>3TC</th>
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<tr>
<td>20-30kg</td>
<td>1.85</td>
<td>1.77</td>
<td>1.00</td>
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**Note:** C24h, C(τ), and C0-24h presented as mean (range).

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946 Pharmacokinetics of Once-Daily DTG/3TC FDC in Children Living With HIV: D3/PENTA21 Sub-Study

Lisanne Bevers1, Man Chan2, Dickson Bwaye3, Adedotu K. Kekitiinwa4, Meurice Alain1, Rashmi Mehta5, Hardik Chandasana6, Sven C. van Dijkman7, Edward Acosta8, Patricia M. Flynn9, Tim R. Cresssey10, Svetlana R. Kekitiinwa1, Kristina M. Brooks1, for the IMPAACT 2019 Protocol Team

**Background:** There is an increasing emphasis on reducing toxicity and improving acceptability of HIV treatment. Two-drug regimens, consisting of two different antiretroviral drug classes, are recommended as an alternative to standard three-drug regimens, with dolutegravir and lamivudine (DTG/3TC) included in a number of adult treatment guidelines. This nested pharmacokinetics (PK) sub-study within the D3/Penta21 randomized controlled trial (NCT04337450) is evaluating DTG and 3TC concentrations in children and adolescents, previously suppressed on a three-drug first-line regimen, who switched to once-daily DTG/3TC fixed dose combinations (FDC).

**Methods:** Eligible children aged 2 to 15 years weighing ≤<40kg received either 5/30mg DTG/3TC dispersible tablets (DT) or 30/500mg film-coated tablets (FCT). Children in weightband (WB) 10-<14kg took 20/120mg DT; WB 14-<20kg 25/150mg DT; WB 20-<25kg either 30/180mg DT or 50/150mg FCT; WB 25-<40kg took 50/150mg FCT, respectively. We aimed for ≥8 evaluable PK curves per WB/formulation. Seven blood samples were taken at steady-state after an overnight fast at t=0, and 1, 2, 3, 4, 6, and 24 h post dosing, PK parameters for DTG and 3TC and the number of children with DTG Ctrough below EC50 (0.32 mg/L) and PA-IC50 (0.064 mg/L) were summarised.

**Results:** 53 participants were included in this preliminary PK analysis (~70% of sub-study participants with successful PK sampling). Median(range) age was 7.1(2.8-13.8) years and weight 7.1(2.8-13.8) kg. DTG Ctrough, AUC(0-24) geometric mean (GM) (95% CI) were 0.71(0.52) mg/L, 64.32(45.1) mg*h/mL and 6.38(3.1) mg/L, 3TC Ctrough, AUC(0-24) and 3TC GM(%) were 0.06(0.35) mg/L, 17.18(6.7) mg*h/mL and 3.25(2.7) mg/L. Figure shows PK parameters by WB. Only 3 children had DTG Ctrough <0.32 mg/L (1 in WB 10-<14kg; 2 in WB 25-<40kg). All children had C24h GM(%CV) were 0.064(9.3) mg/L. In the 20-<25kg WB, 3TC AUC(0-24) GM was 1.5 times higher in children receiving 50/300mg FCT versus 30/180mg DT; however, the AUC(0-24) GM was not much higher than the AUC(0-24) GM in participants weighing ≥25kg.

**Conclusion:** Preliminary PK results demonstrate that DTG and 3TC concentrations after administration of a DTG/3TC FDC in virologically suppressed children living with HIV are comparable with previous paediatric PK studies of DTG (ODYSSEY) and 3TC (IMPAACT2019). As expected, because of a higher dose/kg in the 20-25kg WB in the FCT group in D3/Penta 21 compared to IMPAACT2019 (300mg in 180mg), increased 3TC exposures were observed. Further PK data and safety data are awaited.
Pediatric PBPK Scaling Model for a New Long-Acting, 3 HIV Drug Combination in a Single Injection

Simone Perazzolo, Zachary R. Stephen, Rachele Delle Fratte, Rachel A. Bender
Ignacio, Christine Jonsson, Matthew Hartmann, Pablo Beluazaranz-Zamudio, Keith W. Crawford, Brett Hanscom, Ann C. Collier, Ann J. Melvin, Rodney J. Ho
1University of Washington, Seattle, WA, USA, 2National Institute of Allergy and Infectious Diseases, Rockville, MD, USA, 3Fred Hutchinson Cancer Center, Seattle, WA, USA, 4Seattle Children’s Hospital, Seattle, WA, USA

Background: The TLC-ART program successfully engineered tipranavir (LPV), ritonavir (RTV), and tenofovir (TFV) in a single 3-drug long-acting (LA) injectable suspension (TLC-ART 101) that has undergone scale-up, stability and preclinical safety studies. A first-in-Human study (NCT06850728) is ongoing. Leveraging juvenile and adult non-human primate (NHP) data, we developed and validated physiologically based pharmacokinetic models (PBPK) scalable to inform pediatric dosing of TLC-ART 101.

Methods: PBPK models have been validated with DcNP and the combined free drugs in adult and juvenile NHPs. We scaled the proposed pediatric doses iteratively and simulated time-courses in each age band to predict safe, detectable, and likely effective drug levels in plasma based on combinatorial EC50, AUC, terminal half-life, and concentration at 4 weeks after injection were computed. To scale to children, PBPK accounted for liver CYP3A ontogeny, organ volumes and blood flows, GFR maturation, and drug-drug interactions (DDI).

Results: The projected dose is presented for each pediatric age-weight band according to the dose-equivalent needed to provide target drug exposure (AUC0) for each of the 3 antiretrovirals (LPV/RTV/TFV) in monthly dosing. The apparent half-life for each drug in TLC-ART 101 ranged from 50-300 hours and the weight and metabolic scaling among age groups, were 0.65 to 15.6 mg for LPV, 0.34 to 4.1 mg RTV, and 0.57 to 9.2 mg TFV (~12-24 fold across age bands). Furthermore, possible DDI effects on LPV pharmacokinetics by RTV, which had been observed in NHPs during chronic dosing of TLC-ART 101, were not predicted by the models with the current dosages in humans.

Conclusion: We developed a novel PBPK modeling approach that accounts for anatomical, physiological and enzymatic differences between species and age bands, including DDI assessment due to LPV-RTV. This integrated PBPK model may inform dose selection for clinical evaluation of long-acting HIV drug combinations such as TLC-ART 101, thereby accelerating pediatric access to long-acting treatments. To our knowledge, this drug-combination PBPK model is the first to demonstrate adaptability for multiple drugs as synchronized injections, integrating age-specific physiologic parameters.

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Bone Mineral Density in Children With HIV-1 Receiving TAF-Based Antiretroviral Therapy

N Rakhmanina, Aditya Gaar, Jaime G. Deville, Pope Kosalakaara, Renate Streifauer, Eva Nakaunila, Elizabeth Castati, Vinicius A. Vieira, Yori Kersey, Rory Leisengang, Susanne Crowe, Catherine Gordon
1George Washington University, Washington, DC, USA, 2St Jude Children’s Research Hospital, Memphis, TN, USA, 3University of California, Los Angeles, CA, USA, 4Khanfari University, Khon Kaen, Thailand, 5University of the Witwatersrand, Johannesburg, South Africa, 6Joint Clinical Research Centre, Kampala, Uganda, 7Hospital del Niño, Panama City, Panama, 8Galmed Sciences, Inc, Foster City, CA, USA, 9Baylors College of Medicine, Houston, TX, USA

Background: Some antiretroviral agents, including tenofovir (TFV), may negatively impact bone health. Tenofovir alafenamide (TAF) results in lower TFV plasma levels than tenofovir disoproxil fumarate, and thus has a better safety profile. We examined the medium/long-term treatment effects of TAF-based antiretroviral therapies (ARTs) on bone safety in children and adolescents with HIV aged ≥2 y and weighing ≥14 kg.

Methods: Data from two open-label, multicohort studies including ART-naïve or -experienced pediatric participants with HIV, treated with emtricitabine/TAF-containing ARTs, were pooled by age–weight band: Group (G)1: 12–<18 y, ≥35 kg; G2: 6–<12 y, ≥35 kg; G3: ≥2 y, 14–<25 kg. Bone mineral density (BMD) was assessed using dual-energy X-ray absorptiometry of the spine and total body less head (TBLH) and compared with measurements from individuals without HIV using height–age (HA)–adjusted BMD z-scores.

Results: Overall, 169 participants were treated (G1:78; G2:61; G3:30). For G1, G2 and G3, median ages were 14, 10 and 7 y; 51%, 57% and 63% were female; 72%, 67% and 87% were Black; and 18%, 8% and 3% were Hispanic, respectively. At baseline (BL), the percentage of participants with HIV RNA <50 copies/mL were 35% (G1) and 100% (G2 and G3). Spine and TBLH BMD increased over time (Figure). Spine and TBLH HA-adjusted z-scores also increased from BL, except for a decrease in TBLH HA z-score in female participants in G3. A >4% decrease from BL in spine BMD was observed in 0/44 children in G1 at Week (W) 288, 1/33 in G2 at W240 and 0/25 in G3 at W144. At those timepoints, no participant in any group had a decrease >4% from BL in TBLH BMD. We did not observe any significant correlations between change in HA-adjusted z-score of spine or TBLH BMD at W48 versus TFV maximum plasma concentration or area under the concentration–time curve derived from a noncompartmental analysis over the dosing interval for any of the groups.

Conclusion: These medium/long-term BMD data are reassuring regarding bone safety of TAF-containing ARTs in pediatric participants weighing ≥14 kg. Increases in spine and TBLH BMD were consistent with those observed in children and adolescents without HIV, suggesting that TAF-containing ARTs do not compromise the expected bone accrual in pediatric populations.

Methods: Participants switched from pre-study antiretrovirals to adolescent/adult dosing of CAB-LAI plus RPV-LAI after oral lead-in MOCHA Cohort 2. They were queried about their preferred choice of treatment (every 8-week LAI versus daily oral) and the reasons for this preference at 8- (n=142), 24- (n=141), and 48-weeks (n=115). Reasons for the preferred regimen were recorded verbatim and coded thematically by the study team. In-depth interviews (IDIs)

IMPACT 2017 Adolescent/Parent Experiences With LA Cabotegravir Plus Rilpivirine for HIV Treatment

1University of Pennsylvania, Philadelphia, PA, USA, 2Children’s Hospital of Philadelphia, Philadelphia, PA, 3Frontier Science & Technology Research Foundation, Inc, Amherst, NY, USA, 4Emory University, Atlanta, GA, USA, 5Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 6VII Healthcare, Madrid, Spain, 7Janssen Research & Development, LLC, Pennington, NJ, USA, 8National Institute of Allergy and Infectious Diseases, Rockville, MD, USA, 9FH+10, Bangkok, Thailand, 10University of Alabama at Birmingham–CIRD2, Tuska, Zambia, 11St Jude Children’s Research Hospital, Memphis, TN, USA

Background: The ongoing More Options for Children and Adolescents Study (MOCA; IMPACT 2017; Clinicaltrials.gov NCT03497676) is the first to examine use of long-acting injectable (LAI) cabotegravir (CAB) plus rilpivirine (RPV) in virologically suppressed adolescents, 12 to <18 years of age, with HIV-1. Little is known about the acceptability of this treatment approach for adolescents, including whether it changes over time.

Methods: Participants switched from pre-study antiretrovirals to adolescent/adult dosing of CAB-LAI plus RPV-LAI after oral lead-in MOCHA Cohort 2. They were queried about their preferred choice of treatment (every 8-week LAI versus daily oral) and the reasons for this preference at 8- (n=142), 24- (n=141), and 48-weeks (n=115). Reasons for the preferred regimen were recorded verbatim and coded thematically by the study team. In-depth interviews (IDIs)
were conducted by phone with 8 U.S.-based adolescents and separately with 4 parents after at least 24 weeks on study to provide insight into participants' experiences. Interview transcripts were coded and analyzed using the Consolidated Framework for Implementation Research.

**Results:** Overall, 144 adolescents enrolled in Cohort 2 at 18 sites in 5 countries. All but 4/412 (3%) participants at week 8 and 2/411 (1%) at week 24 stated that they preferred injectable LA medicines over daily orals. The primary themes for preferring LAL were: convenience and burden reduction. The most prominent components of burden reduction were the decrease in adherence-related stress and increased privacy. IDI participants expanded on these themes. All interviewees (adolescents and parents) favored LAL and reported convenience as a driving factor for them/their child to continue the LAL regimen. Having their medical team's support and monitoring for adherence to each LAL dose was repeatedly raised as a key reducer of perceived treatment burden, as was freedom from the daily reminder of HIV diagnosis seen as inherent to oral treatment. A surprising element of the IDI data was feedback that several adolescents had not understood vital elements of the LAL process such as the location of the injections needing to be in the buttocks, despite having completed informed consent/assent counseling.

**Conclusion:** Feedback from adolescents receiving LAL antiretrovirals for up to 48 weeks in the MOCHA study has been favorable thus far. IDIs data suggest that structured and developmentally tailored counseling may be essential to LAL implementation for adolescents.

**950** 
**CABNefewgravi PopPK Analysis of Adolescents & Adults Living With/At Risk for HIV Receiving PrEP**

Yu-Wei Lin,1 S. Y. Amy Cheung1, Isabelle Deperesz1, Susan Ford1, Jen Collins1, Cindy McCoig1, Conn M. Harrington1, Aditya Gaur1, Carolyn Bolton1, Lynda Strainx-Chibanda1, Sybil Hosek1, Mark Marzinkle1, Brookie Best1, Edmund Capparelli1 for the IMPAACT 2017 Team

1Glasnevin Children’s Hospital, Dublin, Ireland, 2ViiV Healthcare, Durham, NC, USA, 3ViiV Healthcare, Madrid, Spain. 4St Jude Children’s Research Hospital, Memphis, TN, USA, 5Center for Infectious Disease Research in Zambia, Lusaka, Zambia, 6University of Zimbabwe, Harare, Zimbabwe, 7John H. Stroger Jr. Hospital of Cook County, Chicago, IL, USA, 8The Johns Hopkins University, Baltimore, MD, USA, 9University of California San Diego, San Diego, CA, USA

**Background:** Cabotegravir (CAB) is an integrase strand transfer inhibitor approved in adults and adolescents (12 to <18 years) weighing ≥35kg as long-acting injectable (LAI) HIV-1 prevention, and for treatment in combination with rilpivirine. An existing CAB population pharmacokinetic (PopPK) model was limited to adult PK (Han 2023). We set out to extend and optimize that existing PopPK model for adolescents (12 to <18 years) by incorporating available CAB PK data following oral lead-in (30mg once daily, QD for at least 4 weeks) and LAI treatment (an initial 600mg 4-week loading dose followed by 900mg Q4W or 600mg Q8W) from 147 adolescents with HIV (IMPAACT 2017) weeks) and LAI treatment (an initial 600mg 4-week loading dose followed by 900mg Q4W or 600mg Q8W).

**Methods:** We investigated the landscape in infants with perinatal HIV from Maputo, Mozambique using multi-omic single cell analyses and 1 million PBMC. 4 HEI (HIV Exposed Infected), ART-naive infants with plasma virus load (VL) between 5.2 and 7.6 logs were evaluated along with 3 HEI (HIV Exposed Uninfected) infants. Cryopreserved PBMC from blood samples collected at 1 mo of age were thawed and cultured with or without anti-CD3 and anti-CD28 Abs for 16-18 hr across 3 independent experiments. After culture, cells were labeled with TotalSeq-c Universal cocktail Abs (Biolegend) for feature barcoding, followed by single cell capture using Chromium (10X Genomics). Libraries were generated for sequencing of m’ RNAseq, surface protein, and V(D)J genes. Sequencing data was mapped and aligned to human reference genome via Cell Ranger (v3.1.2). Quality control, normalization, integration, clustering, and annotation were performed in R using Rdkit, Seurat, and Fastmnn. V(D)J analysis was analyzed with scReptoire.

**Results:** miRNA data generated 30 cell clusters which were manually annotated using a combination of gene and protein expression. Two distinct clusters were identified only in HEI infants: an NK and CD6 + T cell populations. The unique NK subset was characterized by low expression of both CD56 and CD16 by gene and protein expression compared to 3 other NK cell clusters. Relative gene expression analysis revealed downregulation of NK receptors (KLRC2, KLRC4, KLRE1) and cytokines and cytolytic transcripts (IFNG, GENE, GZMA, PMN1) compared to other NK cells, suggesting reduced functional capacity. The enriched CD6 + T cell population in HEI constituted 6% of cells analyzed per donor (median, IQR 1.4-17.4) compared to 0.3% of cells from HEU and V(D)J analysis showed clonal hyperexpansion in this cluster only. These cells were characterized as cytotoxic T lymphocytes (CTL) effectors based on NK-like transcriptomes and high expression of CD57, a marker of terminal differentiation. CTL also expressed FGFR3A/CD16 suggesting potential for ADCC function.

**Conclusion:** Perinatal HIV infection is associated with expanded CTL and NK cells in early life that may play an important role in establishment or shaping of the HIV reservoir.

**952** 
**Distinct Populations of HIV-Infected Naive and Memory CD4+ T-Cell Clones in Children on ART**

Victoria Neer1, Mary Grace Katsuimi1, Shuang Guo1, Sean Patro2, Wenjie Wang1, Xiaolin Wu1, Anna Horner1, Ann Chahroudi1, 2Jason W. Rausch, Maud Mavigner3, Mary F. Kearney1

1National Cancer Institute, Frederick, MD, USA 2Lexis BioMedical Research, Inc, Frederick, MD, USA, 3Emory University, Atlanta, GA, USA

**Background:** Pediatric HIV remains a major public health issue with 1.7 million children living with HIV (CLWH) worldwide. The key obstacle to cure HIV infection is a reservoir of latently infected CD4+ T cells that persist despite long-term antiretroviral therapy (ART). Although HIV primarily infects memory CD4+ T cells, recent studies suggest that naive CD4+ T cells are a significant...
953 Persistent Viremia With vpr/tat-Deleted HIV and No T-Cell Responses to HLA-Predicted HIV Peptides

Alyssa R. Oloidy1, Mariam Azziz2, Sheila Styrchak1, Lennie Chen3, Wenjie Deng1, Melanie Gasper1, Marley Bishop1, Cooper James4, Mih-Suk5, Ewelina Kosmider3, Madeleine Aby1, Lynda Stranix-Chibanda1, Mariam Aziz2, Guinevere Q. Lee2, Xin Pan3, Christian Renaud5, Cooper James4, Mi-Suk5, Ewelina Kosmider3, Madeleine Aby1, Lynda Stranix-Chibanda1, Mariam Aziz2, Guinevere Q. Lee2, Xin Pan3, Christian Renaud5, Cooper James4, Mi-Suk5, Ewelina Kosmider3, Madeleine Aby1, Lynda Stranix-Chibanda1, Mariam Aziz2, Guinevere Q. Lee2, Xin Pan3, Christian Renaud5, Cooper James4, Mi-Suk5, Ewelina Kosmider3, Madeleine Aby1, Lynda Stranix-Chibanda1, Mariam Aziz2, Guinevere Q. Lee2, Xin Pan3, Christian Renaud5, Cooper James4, Mi-Suk5, Ewelina Kosmider3, Madeleine Aby1, Lynda Stranix-Chibanda1

Background: Two children, P1 and P2, living with HIV have persistent low-level viremia (LLV) despite adherence to ART documented by directly-observed-therapy and/or therapeutic drug monitoring. We hypothesized that their persistent viremia was due to clones of HIV-infected cells harboring HIV epitopes that escaped immune surveillance.

Methods: Plasma HIV RNA was monitored. Blood RNA and DNA from P1 over 7 years and P2 over 6 years was used to generate HIV sequences (env, pol, HIV S' and 3'-half and 3Kb central region genomes), to determine HIV integration sites (HIVIS), and to evaluate immunologic biomarkers and HIV-specific immune responses.

Results: Plasma HIV RNA levels ranged from 79-1796c/mL in P1 and 440-15600c/mL in P2, with levels decreasing over time although neither had specimens with undetectable viral loads. No drug resistance was detected in P1, and no novel mutations were detected in 73 single-genome-sequences (SGS) from P2 post immigration to the USA. C2V5 env SGS from P1 and P2 revealed no evidence of HIV evolution over time. P1’s 448 sequences and P2’s 816 sequences revealed 69 and 175 unique indels in the vpr and/or tat1 sequences, with 447/448 (99.8%) and 809/816 (99.3%) genomes having defects in one or both genes. In contrast, 0% of vpr and 0.5% of tat1 genes were defective in 394 DNA sequences from 4 individuals matched for ART-suppression. Elevated immune biomarkers included C-reactive protein and myeloid-derived suppressor cells in P1. The largest HIV cell clones comprised 5% of P1’s 235 and 3% of P2’s 287 HIVIS. Interferon-gamma ELISPots with (1) non-escaped predicted HIV HLA-binding peptides (76 for P1 and 25 for P2), (2) HIV gag, Pol, and nef potential T-cell epitope peptide pools from the N1H and (3) control antigens, detected reactive T cells to control antigens, but no reactive T cells to HIV (1) or (2) in P1 or P2 across multiple timepoints.

Conclusion: Our data suggest that the persistent LLV in these two individuals is unlikely to have originated from proliferating HIV-infected clones or from ongoing HIV replication. Rather, their lack of HIV-specific T cell responses suggests that ongoing selection maintains the cells with vpr- and tat1-deleted viruses, possibly by conferring resistance to apoptosis.

954 Proviral Landscape in Neonates With In Utero HIV-1 on Very Early ART in IMPAACT P1115

Soumia Bekka1, Priya R. Khetan1, Yufeng Liu2, Adit Dhummakupt1, Bryan S. Nelson3, Camlin Tierney4, Ellen G. Chadwick1, Jennifer Jao5, Yvonne Bryson6, Anne Coletti7, Nicol Nicodimus8, Lynda Stranix-Chibanda1, Guinevere Q. Lee2, Stuart Ray2, Deborah Persaud1

The cohort consisted of 8 children aged 5-11 years who initiated antiretroviral treatment (ART) within 48 hours of birth in the IMPAACT P1115 study.

Methods: 21 neonates with in utero HIV-1 in IMPAACT P1115 met criteria for near full-length single genome sequencing (nFLSGS) at either Step 1 (within 48 hours post-birth; N=16), Step 2 (7-18 days post-birth when infection was confirmed; N=17) or both (N=12). Of the 13 neonates excluded at either timepoint, 11 had low proviral DNA loads (<20 copies/10^6 PBMCs) and 2 had low sample volumes (<5 ul) insufficient for nFLSGS amplification. nFLSGS was performed using a standard input of HIV-1 DNA per neonate to ensure single proviral genome amplification. nFLSGS data were classified with HIVSeqinR as intact, defective, or hypermutated, and copy numbers were standardized and efficiency adjusted to c/10^6 PBMCs. The limit of detection was set to 0.5 copies per number of cells assayed. ART drug resistance was evaluated using the Stanford HIV Drug Resistance Database.

Results: In the 21 neonates analyzed, median log10 HIV-1 DNA load was 2.8 at Step 1 and 2.9 at Step 2 (median 15 days). nFLSGS examined 93 genomes at Step 1 and 111 genomes at Step 2 (median 6 genomes per neonate in median 50000 cells). At our sampling depth, we detected intact proviruses in 13/16 (81%) neonates at Step 1 and 14/17 (82%) at Step 2. Intact proviruses comprised a median of 50% of each neonate’s observed genomes at Step 1 and 33% at Step 2. Median log10 intact proviral load was 2.0 at Step 1. Among the 12 neonates with data at both timepoints (median 14 days apart), intact proviruses shared >99.8% sequence identity. Defective and hypermutated proviruses were detected at either timepoint in 20/21 and 9/21 neonates. The K10QN resistance mutation was detected in all intact proviruses at both timepoints for one neonate, confirmed with maternal genotyping. No other major resistance mutations were identified in the 21 neonates.

Conclusion: The proviral landscape at birth of neonates with in utero HIV-1 and quantifiable HIV-1 DNA includes a mix of intact, defective, and hypermutated proviruses, indicating in utero HIV-1 replication. Intact proviruses share >99.8% sequence identity through Step 2. These findings suggest early formation of abundant intact HIV-1 DNA populations, which could establish reservoirs that pose challenges to HIV-1 remission.

955 CMV Coinfection Drives T-Cell Differentiation but Not Reservoir Size Among Children With HIV

Yves Fougère1, Jason Brophy2, Ari Bitun3, Michael T. Hawkes4, Linda Samson5, Mi-Suk Kang Dufoz5, Christian Renaud4, Stanley Read1, Hanita K. Dieumegard1, Madeleine Aby Diallo1, Jade Canape1, Soren Gant1, Hugo Soudiemy2, Fatima Kakkar2

The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, Harvard TH Chan School of Public Health, Boston, MA, USA, Northwestern University, Chicago, IL, USA, University of California Los Angeles, Los Angeles, CA, USA, THI, Durham, NC, USA, University of Zimbabwe, Harare, Zimbabwe, Weill Cornell Medicine, New York, NY, USA

Background: We studied the proviral landscape of neonates with in utero HIV-1 who initiated antiretroviral treatment (ART) within 48 hours of birth in the IMPAACT P1115 study.

Methods: 21 neonates with in utero HIV-1 in IMPAACT P1115 met criteria for near full-length single genome sequencing (nFLSGS) at Step 1 within 48 hours post-birth (N=16), Step 2 (7-18 days post-birth when infection was confirmed; N=17) or both (N=12). Of the 13 neonates excluded at either timepoint, 11 had low proviral DNA loads (<20 copies/10^6 PBMCs) and 2 had low sample volumes (<5 ul) insufficient for nFLSGS amplification. nFLSGS was performed using a standard input of HIV-1 DNA per neonate to ensure single proviral genome amplification. nFLSGS data were classified with HIVSeqinR as intact, defective, or hypermutated, and copy numbers were standardized and efficiency adjusted to c/10^6 PBMCs. The limit of detection was set to 0.5 copies per number of cells assayed. ART drug resistance was evaluated using the Stanford HIV Drug Resistance Database.

Results: In the 21 neonates analyzed, median log10 HIV-1 DNA load was 2.8 at Step 1 and 2.9 at Step 2 (median 15 days). nFLSGS examined 93 genomes at Step 1 and 111 genomes at Step 2 (median 6 genomes per neonate in median 50000 cells). At our sampling depth, we detected intact proviruses in 13/16 (81%) neonates at Step 1 and 14/17 (82%) at Step 2. Intact proviruses comprised a median of 50% of each neonate’s observed genomes at Step 1 and 33% at Step 2. Median log10 intact proviral load was 2.0 at Step 1. Among the 12 neonates with data at both timepoints (median 14 days apart), intact proviruses shared >99.8% sequence identity. Defective and hypermutated proviruses were detected at either timepoint in 20/21 and 9/21 neonates. The K10QN resistance mutation was detected in all intact proviruses at both timepoints for one neonate, confirmed with maternal genotyping. No other major resistance mutations were identified in the 21 neonates.

Conclusion: The proviral landscape at birth of neonates with in utero HIV-1 and quantifiable HIV-1 DNA includes a mix of intact, defective, and hypermutated proviruses, indicating in utero HIV-1 replication. Intact proviruses share >99.8% sequence identity through Step 2. These findings suggest early formation of abundant intact HIV-1 DNA populations, which could establish reservoirs that pose challenges to HIV-1 remission.
Results: Of 226 CLWH enrolled in EPIC4, 108 met sub-study inclusion criteria; of these, 82.4% were CMV+ at baseline. There were no significant differences in total HIV-1 DNA (median = 1.70 vs 1.71 log10 HIV DNA copies per 10^6 cells) or inducible cell-free HIV-1 RNA (median = 0.79 vs 0.60 log10 HIV RNA copies per 10^6 cells) between CMV+ and CMV- participants, both on univariate and multivariate analysis adjusting for age, sex, at treatment initiation, duration of VS, and peak lifetime HIV viral load. However, while frequencies of total CD8+ and CD8+ central memory (TCM) cells were similar among CMV+ and CMV- children (39.7% vs 31.8%, and 2.5 vs 2.9%, respectively), CMV+ children exhibited significantly lower frequencies of CD8+ T naive (TN) cells (54.6 vs 70.9%, p = 0.003), and significantly higher frequencies of CD8+ T effector memory (TEM) cells (6.8 vs 4.6%, p = 0.047) and CD8+ T terminally differentiated effector (TEMRA) cells (19.5 vs 9.6%, p = 0.003). No differences in total, TN or TCM cell frequencies were observed in CD4+ T cells between CMV+ and CMV- participants, but CMV+ participants had a significantly higher frequency of CD4+ TEM cells (5.7 vs 3.7%, p = 0.005). This difference remained significant after adjustment for age, sex at treatment initiation, and age at HIV viral suppression. Conclusion: CMV co-infection was not significantly associated with HIV reservoir size in CLWH, but significantly affected the naive-memory-effector profile of CD4+ and CD8+ T cells. These results suggest CMV co-infection may alter the differentiation and maturation of CD4+ and CD8+ T cells in CLWH independent of achievement of VS.

956 Reservoir Size and Dynamics by Age at Virologic Suppression and Sex in Youth With Perinatal HIV-1
Priya R. Khetan, Wendy Yu, Kunjal Patel, Joseph Sz ewczyk, Adit Dhummakupt, Sandra Burchett, Russell Van Dyke, Deborah Persaud, for the Pediatric HIV/AIDS Cohort Study (PHACS)
The Johns Hopkins University School of Medicine, Baltimore, MD, USA, ’Harvard TH Chan School of Public Health, Boston, MA, USA,’Boston Children’s Hospital, Boston, MA, USA,’Tulane University, New Orleans, LA, USA

Background: Studies on HIV-1 reservoir size and dynamics in youth with perinatal HIV-1 with long-term viral suppression (VS) are limited. Methods: We identified longitudinal peripheral blood mononuclear cells (PBMC) specimens among youth with perinatal HIV-1 in the PHACS AMP study who were early-suppressed (ES) at <1 year of age or late-suppressed (LS) at 1-5 years of age and maintained VS. LS were matched to ES by age at last specimen, percent without viral blips, and sex. HIV-1 reservoir size was assessed with the Intact Proviral DNA assay (IPDA). Data were summarized by age at VS, sex, and duration of VS. HIV-1 DNA slopes over follow-up were estimated using linear mixed-effects models. Results: We included 11 ES (6 females, 5 males) and 15 LS (7 females, 8 males) with a median of 8 specimens per participant over a median of 17.3 years of VS. Median age at VS and duration of VS were 0.6 and 17.6 years for ES, and 2.7 and 16.5 years for LS. PBMCs were primarily collected from 5 to <15 years of VS. The median limit of detection for the IPDA with a median of 500,000 cells analyzed was 2 copies/million PBMCs. Total and intact proviruses were lower in ES compared to LS (Figure 1). From 5 to <10 and 10 to <15 years of VS, median total HIV-1 DNA was 20.0 and 28.0 copies/million PBMCs in ES compared to 113.2 and 103.3 copies/million PBMCs in LS. Median intact HIV-1 DNA from 5 to <15 years of VS was undetectable in ES, and detectable at 6.3-6.4 copies/million PBMCs in LS. Median intact HIV-1 DNA from 5 to <15 years of VS was undetectable in ES, and detectable at 6.3-6.4 copies/million PBMCs in LS. Median intact HIV-1 DNA from 5 to <15 years of VS was lower from 5 to <10 years and higher from 10 to <15 years of VS compared to LS males. LS females had higher median total HIV-1 DNA from 5 to <15 years of VS compared to LS males. Median intact HIV-1 DNA was undetectable for both ES females and males. However, LS females had higher median intact HIV-1 DNA from 5 to <15 years of VS compared to LS males. Estimated mean slopes of total HIV-1 DNA from 5 to <15 years were similar by sex among ES but differed among LS: total HIV-1 DNA increased by 2.4% per year for females and decreased by 5.6% per year for males. Mean slopes of intact HIV-1 DNA for ES females, ES males, and LS males were uncertain due to a high percent of undetectable values, complicating comparisons by sex. Intact HIV-1 DNA decreased over VS for LS females.

Conclusion: We observed HIV-1 reservoir size to be smaller by age 5 years with early VS maintained through young adulthood. Among LS youth, we identified sex differences in the reservoir size with relevance to ART-free remission.

957 Latency Reversal Effects of TLR7 Agonist and HDACi in Long-Standing Perinatal HIV-1 Infection
Kristen E. Kelly, Adit Dhummakupt, Joseph Szewczyk, Wei-qiang Zhou, Hongkai Ji, Ya Hui Chen, Thuy Anderson, Elise T. Ohene-Kyei, Allison Agwal, Deborah Persaud, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, ’The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: Latency reversing agents (LRAs) such as TLR7 agonist and histone deacetylase inhibitors (HDACi) reactivate proviral latency in adults. We examined the LRA effects of the TLR7 agonist, GS-9670 singly and in combination with the HDACi, SAHA, in a cohort of children and young adults living with perinatal HIV-1 (CYPALPH) to inform latency reversal strategies for this population. Methods: Peripheral blood mononuclear cells (PBMCs) and purified CD4 T cells from a cohort of 8 CYPALPH, median age 17.0[iqr, 16.7-20.3] yrs, with median virologic suppression of 13.3[8.2-16.3] yrs were stimulated ex-vivo with GS-9620 singly and in combination with SAHA for 56 hrs. Concomitant stimulation with PFA/PNA/Ionomycin (PPI) and DMSO were performed to assess proviral reactivation under maximal T cell stimulation and vehicle control. Proviral reactivation was determined by multiply spliced HIV-1 RNA (msRUPM) with the Tat/Rev Limiting Dilution Assay. Total, intact and defective proviral HIV-1 DNA was quantified by multiplexed ddPCR; cell-surface marker and histone acetylation expression by flow cytometry, culture supernatant for 9 cytokines with the MSD platform and p24 by SIMOA.

Results: The median total HIV-1 DNA load was 262[iqr, 52-485.2] copies per million(cpm) CD4s; the 5 subtype B participants had 130-261.1 intact cpm, 4.96% of total HIV-1 DNA. PBMCs stimulated with GS-9620 plus SAHA showed reactivation in only 2 of 6 participants. In CD4 T cells, PPI stimulation increased msRUPM in 8(75%) participants to 13.71±2.04; with DMSO, msRUPM was 6.04[3.4-10.6]. With GS-9620 and SAHA singly, 5(63%) and 4(50%) participants exhibited reactivation, respectively. With dual stimulation, reactivation was detected in 5 participants. Median msRUPM fold change to DMSO was 1.5[0.97-2.34] for PPI, 1.33[0.78-1.93] for GS-9620, 0.76[0-1.52] for SAHA, and 1.22[0.76-1.56] for GS-9620 plus SAHA. One participant, durably suppressed since infancy, had no reactivation, and one participant displayed inhibition of ms-transcripts under all conditions. PPI stimulation was associated with upregulation of CD69, CD25, and HLA-DR whereas only CD69 was upregulated in SAHA conditions. Secreted IL-6 was higher after GS-9620 stimulation compared to DMSO. Histone acetylation effects were seen in 5 participants after SAHA treatment.

Conclusion: These findings suggest that pediatric reservoirs can be reactivated with innate immune enhancers, such as TLR agonist and HDACi, with implications for use as therapeutics to purge HIV-1 reservoirs.
Predictive Markers for Sustained Viral Suppression on Dual bNAbs During ART Interruption in Children


1Beth Israel Deaconess Medical Center, Boston, MA, USA, 2Harvard Th Chan School of Public Health, Boston, MA, USA, 3Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 4Massachusetts General Hospital, Boston, MA, USA, 5Brigham and Women’s Hospital, Boston, MA, USA, 6Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA

Background: Phenotyping to identify susceptibility to broadly neutralizing antibodies (bNAbs) is costly and logistically challenging, and simple clinical markers to predict bNAb treatment success for children are needed. The combination of a negative qualitative HIV DNA and negative enzyme immunoassay for an HIV RNA target detection below the limit of detection at study entry was previously reported to correlate with success in the Tateto Study in Botswana. We now report findings for additional biomarker combinations at study entry and during the bNAb-only step of the study.

Methods: Twenty-five children received up to 24 weeks of bNAb-only treatment (VRC01/LSI+10-1047) following ART interruption. HIV qualitative DNA and HIV RNA were performed every 1-2 weeks and EIA every 4-8 weeks. Using Fisher’s exact test we compared the proportion of infants who remained virally suppressed to < 400 copies/mL (successes) to those with detectable virus by assay type.

Results: During the bNAb-only step of Tateto there were a median of 8 qualitative HIV DNA measurements, 12 HIV-1 RNA “target detection” measurements, and 4 EIA measurements per participant available for analysis. At bNAb-only step entry, 13/25 (52%) had negative qualitative DNA and 17/25 (68%) had negative EIA, and 10/25 (40%) were negative for both. Nine of the 13 (69%) with negative qualitative DNA succeeded compared with 2/12 (17%) with positive or indeterminate qualitative DNA (p=0.02). Nine of the 17 (53%) with negative EIA succeeded compared with 2/8 (25%) with positive EIA (p=0.23). Combining biomarkers, 8/10 (80%) who were negative/negative at bNAb-only entry succeeded, compared with 3/15 (20%) with any other pattern (p=0.005); all 8 successes (and no failures) were negative/negative at study entry (the bNAb+ART overlap step), as previously reported. HIV-1 RNA “target detection” was below the assay limit in all but one participant at the start of the bNAb-only step. In the visit immediately prior to any viral rebound, target detection occurred in only 1/14 (7%) participants (Figure 1). No marker or combination of markers reliably changed in the 3 visits prior to rebound.

Conclusion: Negative qualitative DNA, and especially negative/negative DNA and EIA, at start of bNAb-only step predicted maintenance of viral suppression among children on dual bNAbs. HIV-1 RNA target detection below the limit of detection at study entry (the bNAb-only step) is a useful clinical marker of treatment success.

Figure 1: Schematic representation of bNAb-only step (top) and the assignment of HIV status at study entry (bottom). (A) Time to viral rebound of ART week. (B) Initial false positive results on the Xpert® platform were 18 (90%) were tested on just 1 of the 6 Xpert® machines; this machine was removed from service and is under evaluation, without an identifiable cause for the false positive results (operator error deemed unlikely). Excluding all 453 samples tested on this machine yields a false positive rate of 0.2% (2/1026), or 15% (2/13) of all positive results. The median cycle threshold (CT) value for false positive results on the Xpert® platform was 39.2 (range 34.5, 45.9) compared with a median of 32.9 (range 24.2, 41.0) for true positives, indicating some overlap between groups (Figure 1).

Conclusion: The Xpert® POCT platform offers affordable and easily implementable birth testing for HIV, but false positive testing may occur. High CT values provide an initial indication of a possible false positive result, but all positive testing must be confirmed by either HIV-1 RNA testing, HIV-1 DNA testing, or both.

Figure 1: Cycle threshold values at birth using Cepheid Xpert® HIV-1 qualitative assay

High Proportion of False-Positive HIV Results With Point-of-Care Birth Testing in Botswana


1Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 2Brigham and Women’s Hospital, Boston, MA, USA, 3Harvard Th Chan School of Public Health, Boston, MA, USA, 4Massachusetts General Hospital, Boston, MA, USA, 5Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA

Background: As countries in Sub-Saharan Africa move toward rollout of point-of-care testing (POCT), risk for false positive testing and pathways to make accurate HIV diagnoses need to be understood.

Methods: The Moso Study identified newborns at high risk of HIV acquisition at 35 delivery facilities in Botswana. High-risk status was defined primarily (but not exclusively) as limited maternal ART duration in pregnancy. HIV testing was performed using the Cepheid Xpert® HIV-1 qualitative POCT platform, with Roche CAP/CTM HIV-1 qualitative confirmation for those testing positive. Further testing with HIV-1 RNA quantification and droplet digital polymerase chain reaction (ddPCR) was conducted for discordant results. Subsequent 6-week HIV test results for infants with negative birth POCT were abstracted from national electronic laboratory.medical records.

Results: From July 2022—August 2023, using 6 Xpert® machines housed in close proximity to the 35 delivery sites, HIV-1 RNA testing was performed for 1479 high-risk newborns were tested for HIV with POCT at a median of 21 hours of life (range 0, 158 hours). Thirty-one newborns (2%) had initial positive results, but only 11 (39%) were confirmed positive by Roche CAP/CTM testing, for an overall vertical transmission rate of 0.7% in high-risk neonates. The 20 (1.4%) false positives were further evaluated by HIV-1 RNA testing (all undetectable < 40 copies/mL); ddPCR testing was performed on 18 (90%) false positive samples with no target detected in any. Follow-up testing of false positives to date has remained negative. Of the 20 initial false positive samples, 18 (90%) were tested on just 1 of the 6 Xpert® machines; this machine was removed from service and is under evaluation, without an identifiable cause for the false positive results (operator error deemed unlikely). Excluding all 453 samples tested on this machine yields a false positive rate of 0.2% (2/1026), or 15% (2/13) of all positive results. The median cycle threshold (CT) value for false positive results on the Xpert® platform was 39.2 (range 34.5, 43.9) compared with a median of 32.9 (range 24.2, 41.0) for true positives, indicating some overlap between groups (Figure 1).

Conclusion: The Xpert® POCT platform offers affordable and easily implementable birth testing for HIV, but false positive testing may occur. High CT values provide an initial indication of a possible false positive result, but all positive testing must be confirmed by either HIV-1 RNA testing, HIV-1 DNA testing, or both.

Figure 1: Cycle threshold values at birth using Cepheid Xpert® HIV-1 qualitative assay

Rapid Antiretroviral Therapy Initiation Following Point-of-Care Early Infant Diagnosis in Uganda

Stella M. Migamba, Tamara N. Nyombi, Edrisa Nsugubi, Andrew Kwiringira, Benon Kwaresga, Steven N. Kabwama, Mary Nakafeero, Daniel Kadobera, Phoibe Mayamba, Lilian Bulago, Alex Ariyo, Julie Harris

1Uganda National Institute of Public Health, Kampala, Uganda, 2Other Institution — Follow-up needed, Uganda, 3Makerere University, Kampala, Uganda, 4Centers for Disease Control and Prevention, Kampala, Uganda

Background: Uganda Ministry of Health recommends a first HIV DNA-PCR test at 4-6 weeks for early infant diagnosis (EID) of HIV-exposed infants (HEIs), immediate results return and initiation of antiretroviral therapy (ART) for infants with HIV infection. In 2019, MOH introduced point-of-care (POC) whole-blood EID testing in 33 health facilities and scaled up to 133 in 2020. We assessed turnaround time for test results and ART linkage before and after implementation of POC testing.

Methods: We evaluated EID register data for HEI at 10 health facilities with POC and minimum EID testing volume of 12 infants per month from 2018-2021. At each facility, we abstracted data for 12 months before and after POC rollout. We compared time to sample collection, results receipt, and ART initiation between periods using medians, Wilcoxon rank-sum, and log-rank tests.
Results: Data for 4,004 HEI were extracted, including 1,688 (42%) pre-POC and 2,316 (58%) during POC. Ninety-four percent of infants (3,762/4,004) had a first EID test. Median age at sample collection was 44 (IQR 38-52) days pre-POC and 42 (IQR 38-52) days during POC (p < 0.001). Of 3,762 HEI tested, 3,667 (97%) had test results. For infants with HIV infection (n = 69), median age at sample collection was 92 (IQR 45-120) days pre-POC and 127 (IQR 74-206) days during POC (p = 0.03). For all infants, median days from sample collection to results receipt by infants’ caregivers were 29 (IQR 16-54) pre-POC and 1 (IQR 0-2) during POC (p < 0.001); among infants with HIV infection, median days were 22 (IQR 4-30) pre-POC and 0 (0-3) during POC (p < 0.001). Pre-POC, 0% (0/23) infants with HIV infection started ART on the sample collection day compared to 40% (17/42) during POC. ART linkage by 7 days after HIV diagnosis was 78% (21/27) pre-POC and 95% (40/42) during POC (p < 0.001).

Conclusion: POC testing improved EID test turnaround time and ART initiation for infants with HIV infection. Later age at testing among infants diagnosed with HIV suggests missed opportunities in identifying HIV-exposed infants. While POC expansion could further improve ART linkage and loss to follow-up, there’s need to examine barriers surrounding the POC target of initiating ART on the sample collection day.

961 Resistance in Young Children Newly-Diagnosed With HIV in Western Cape, South Africa


Background: Pretreatment drug resistance among children living with HIV (CHLV) can potentially compromise antiretroviral therapy (ART) effectiveness. Drug resistant HIV may be directly transmitted during vertical acquisition or may be resistant in infants with HIV following exposure to antiretrovirals consumed through breastfeeding or administered as prophylaxis.

Methods: We performed resistance testing in young CHLV (age < 3 years) newly diagnosed with HIV in Western Cape, South Africa (July 2021 to October 2022) who either (1) acquired HIV via possible breastfeeding transmission from mothers who received ART (any regimen) during pregnancy/postpartum and/or (2) were exposed to protease inhibitors (PIs) or integrase strand transfer inhibitors (INSTIs) in utero. Possible breastfeeding transmission was defined as a positive HIV-PCR test at age >28 days, which occurred after a previous negative HIV-PCR test. We limited mutations included to those recommended for surveillance of transmitted drug resistance.

Results: We included 135 newly diagnosed CHLV for resistance testing, of whom 91% (123) had possible breastfeeding transmission. Most mothers started ART before pregnancy (77%) and were exposed to ≥3 classes of ART prior to infant diagnosis (66%). Overall, 58% of CHLV (78/135) had resistance mutations detected and 15% of those with mutations (12/78) had mutations to more than one class. Non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated mutations were common (33/78, 43%). Nucleoside reverse transcriptase inhibitor- (RTV)- and PI-associated mutations were found in 18% (14/78), 3% (2/78) and 1% (1/78) of CHLV with mutations, respectively. One child with breastfeeding transmission of HIV had high-level INSTI and NNRTI resistance detected at age 18 months at HIV diagnosis (E133K, G118R and K103N mutations). The mother had changed from efavirenz- to dolutegravir-based ART 13 months after delivery.

Conclusion: NNRTI-associated mutations are common and may be transmitted or arise from exposure to NNRTIs as prophylaxis or in breastmilk. Dolutegravir is the preferred first-line treatment for both adults and CHLV older than 4 weeks and very low INSTI resistance levels have been observed in adults, but limited data exist on genotyping the integrase region in CHLV. The prevalence of pretreatment INSTI resistance in CHLV is likely to be very small but future surveillance is necessary. There is a need for further, longitudinal studies with paired mother-infant resistance results.

962 CMV Viraemia Is Associated With Mortality Among Children With HIV Starting ART in Sub-Saharan Africa

Temitope Fisayo, Gert U. van Zyl, Nei-Yuan Hsiao, Florence Phelanyane, Andrew Boulle, Temitope Fisayo, Cissy Kityo, Diana Gbiti, Cissy Kityo, Dietrich Klein, Sarah Walker, Andrew Prendergast, Queen Mary University of London, London, United Kingdom; Anu, South Africa; UCL Great Ormond Street Institute of Child Health, London, United Kingdom; University College London, London, United Kingdom; University of Zimbabwe, Harare, Zimbabwe; Makerere University, Kampala, Uganda; Baylook College of Medicine Children’s Foundation, Kampala, Uganda, Joint Clinical Research Centre, Kampala, Uganda

Background: Cytomegalovirus (CMV) co-infection is associated with mortality in adults with HIV, but the association between CMV viraemia and mortality in children with HIV is uncertain.

Methods: In 497 children enrolled in the ARROW trial (registry number ISRCTN24791884) in Uganda and Zimbabwe, CMV was quantified using real-time polymerase chain reaction (PCR) at antiretroviral therapy (ART) initiation, and after 12 and 84 weeks post-initiation in a case-cohort design. Associations between CMV viraemia and mortality were evaluated using multivariable models, adjusting for HIV viral load, CD4 percentage and inflammatory biomarkers.

Results: CMV viraemia was associated with mortality, but the relationship differed by country and assay type. In Zimbabwe, where a more sensitive assay led to a high prevalence of detectable CMV (73%), each log rise in CMV viral load was associated with 2-fold higher mortality (adjusted hazard ratio (aHR) 2.35; 95% confidence interval (CI) 1.34, 4.12). In Uganda, where the assay sensitivity was lower, children with detectable CMV viraemia (26%) had almost 3-fold higher mortality than children without detectable CMV (aHR 2.86; 95%CI 1.02, 8.33).

Conclusion: CMV viraemia at the time of ART initiation is associated with mortality in children with HIV in sub-Saharan Africa, independent of HIV viral load, immunosuppression and immune activation. Future studies should evaluate whether suppressing CMV viraemia reduces mortality in children with HIV.

963 Sex Differences in Growth Trajectories Between Early-Treated Infants With HIV and Controls

Ana Barrios-Tascon, Renate Streithau, Farezah Patel, Megan Burke, Stephanie Shiavi, Yannan Shen, Stephen M. Arpadul, Elaine J. Abrams, Caroline T. Tiemessen, Louise Kuhn, for the LEOPARD Study Team

Background: Perinatally-acquired HIV infection is associated with increased risk of postnatal growth deficits which are only partially corrected by antiretroviral therapy (ART). Studies of sex differences in immune recovery and virologic response to ART among infants with perinaturally acquired HIV (IHIV) have reported conflicting results. Here we investigate the role of sex in modifying the growth trajectories of IHIV who started ART at an early age.

Methods: As part of an early ART trial conducted in Johannesburg, South Africa (2015-2018), 116 IHIV diagnosed within 48 hours of birth initiated ART as early as possible, consisting of nevirapine (switched to lopinavir-ritonavir >> 42 weeks postmenstrual age) lamivudine, and zidovudine (switched to abacavir at 3 months). Eighty infants born to mothers living with HIV but found to be uninfected (IHEU) were enrolled as controls, receiving daily nevirapine for 6 weeks, and twice-daily zidovudine added if high risk. Both groups were followed prospectively through 48 weeks and anthropometric parameters collected. Age and sex adjusted Z-scores for weight (WAZ) and length (LAZ) were compared between IHIV and IHEU, as well as stratified by sex. Generalized
linear models were used to describe growth trajectories and main and interactive effects.

**Results:** Just under half the infants were male (48.5%), similar proportions in HIV and IHEU. The deficits in growth through 48 weeks associated with HIV status were most marked in female infants (Figure). Mean WAZ (β = -0.51 [standard error 0.22], p = 0.02) and LAZ (β = -0.50 [0.21], p = 0.02) were lower in female HIV than in female IHEU overall (Figure). In contrast, trajectories of WAZ (β = -0.21 [0.30], p = 0.48) and LAZ (β = 0.03 [0.32], p = 0.92) between male HIV and male IHEU were similar. Combining the two groups, males had lower WAZ than females over the 48 week period. Males also had lower LAZ than females, with significant differences at 12 and 24 weeks (-1.84 [0.22] vs -1.34 [0.12], p = 0.04 and -1.53 [0.19] vs -1.01 [0.12], p = 0.02). The mean WAZ and LAZ in neither group attained WHO standards by 48 weeks.

**Conclusion:** Deficits in WAZ and LAZ in HIV with early ART compared to IHEU were greater in girls. This pattern was observed despite boys having consistently lower anthropometric parameters than girls in both groups. Factors responsible for this sex difference are not clear, but early ART initiation and/or perinatal infection appears to have a differential impact on growth according to sex.

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**Figure.** Weight for age z-score (WAZ), and length for age z-score (LAZ) in males and females by HIV status. HIV: infants with HIV; IHEU: infants exposed and not infected by HIV. *Difference p<0.05 between female HIV and IHEU groups.*

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**VI. Causes of Death After Early ART in Infants Living With HIV From 3 Sub-Saharan African Countries**

Alfredo Tagarro, Sheila Fernández-Luis, Sara Dominguez-Rodriguez, Alvaro Ballesteros, Louise Kuhn, Mark F. Cotton, Carlo Giaquinto, Paola Rossi, Maria Grazia Lain, Kennedy Otowome, Osee Behuhuma, Maria Grazia Lain, Pablo Rojo, Anita Janse van Rensburg, Sara Dominguez-Rodriguez, Carlo Giaquinto, for IAS–USA

**Background:** Despite comprising only 4% of all positive people, children account for 15% of all HIV/AIDS-related deaths. Mortality unrelated to AIDS is also high in LMIC. We investigated causes of death, potential relationship with HIV infection, and associated factors in a cohort of early treated children.

**Methods:** From May 2018 to May 2021, we recruited infants who initiated ART within the first 6 months of life and within 3 months of diagnosis. Follow-up was 4 years. There were 6 study sites in South Africa, Mozambique and Mali. HIV/AIDS-related mortality was determined by an independent Endpoint Review Committee comprising three HIV Pediatric infectious disease specialists not directly involved in participant care. The experts assessed the relationship of each death to HIV advanced disease using available epidemiological, clinical, and laboratory data from the study database. Mortality risk factors were analyzed using a competing risk Cox multivariable regression model.

**Results:** Of 215 infants enrolled, the median age at HIV diagnosis was 31 days with ART initiation at a median age of 34 days (IQR 26.0;73.0). Follow-up was 34.0 months (IQR 16.3;44.1). Median VL at ART initiation was log 4.95 (IQR 3.5;8.82) copies/mL. Twenty-five infants (11.6%) died at a median age of 5.3 months (IQR 3.0;9.6). The probability of death within the first year of ART initiation was 12% (95% CI 6-14%), and after 2 and 3 years, 12% (95% CI 8-17%). The primary causes of death were pneumonia (36%), undetermined (24%), tuberculosis (12%), malnutrition (8%), diarrhea (8%), sepsis (8%) and malaria (4%). Death was assigned as likely HIV/AIDS-related in 8/25 (32%) of deaths. VL at ART initiation was significantly associated with all deaths (HIV/AIDS-related cause HR:3.0 (95% CI 1.3-7.1), p = 0.014; unclear relation, HR:1.7 (95%CI 1.1-2.8), p = 0.019). The more positive the CD4 slope, the lower the probability of death in HIV/AIDS-related (HR:0.7 (95%CI 0.5-1.0), p = 0.067) or unrelated (HR:0.7 (95%CI 0.5-0.9), p = 0.007). Patients from Mali had a higher probability of death from HIV/AIDS-unrelated causes, compared to those enrolled in South Africa (HR:13.8 (95%CI 1.92-98.8), p = 0.009).

**Conclusion:** Mortality, both related with HIV/AIDS or probably unrelated, were associated with baseline VL and poor CD4 restoration. However, the 3-fold risk for high baseline VL and HIV/AIDS-related causes supports a strong biological effect of baseline VL along with a challenging social environment.
Usefulness of Clinical Signs for Tuberculosis Diagnosis in Infants Living With HIV With Pneumonia

Tisungane Mvalo,1 Cinta Moraleda,2 William C. Buck,3 Victor Musiime,4 Sara Dominguez-Rodriguez,2 Chishala Chabala,2 Hilda A. Mujuru,2 Alfredo Tagarro,1 Pui-Ying Iroh Tam1, Jahit Sacarlal1, Chishala Chabala3, John Tembo1, Sheila Fernandez-Luiz2, Pablo Rojo,4 for the EMPIRICAL Clinical Trial Group

1University of North Carolina Project–Malawi, Lilongwe, Malawi, 2Hospital Universitario de Octubre, Madrid, Spain, 3University of California Los Angeles, Los Angeles, CA, USA, 4Makerere University, Kampala, Uganda, University of Zambia, Lusaka, Zambia, University of Zimbabwe, Harare, Zimbabwe, Malawi-Liverpool Wellcome Trust Clinical Research Programme, Blantyre, Malawi, Universidade Eduardo Mondlane, Maputo, Mozambique, Jinja Regional Referral Hospital, Jinja, Uganda, 5University Teaching Hospital, Lusaka, Zambia

Background: Children living with HIV are at increased risk of tuberculosis (TB) morbidity and mortality. However, TB diagnosis remains challenging, particularly in infants who have paucibacillary disease and are more likely to have atypical clinical presentations. Consensus case definitions based on microbiological confirmation, signs/symptoms, chest radiograph features, TB exposure/immunological evidence of infection, and response to treatment have been proposed by Graham et al. to standardize the reporting of cases of intrathoracic tuberculosis. We evaluated the prevalence of the Graham clinical criteria (persistent cough, weight loss/failure to thrive, persistent unexplained fever or lethargy, neonatal pneumonia or hepatosplenomegaly) in relation to confirmed TB diagnosis in infants living with HIV hospitalized with severe pneumonia.

Methods: EMPIRICAL is a randomized clinical trial (#NCT03915366) funded by EDCTP (RIA2017MC-2013) investigating the impact of empirical treatment of cytomegalovirus and/or TB treatment on mortality in infants living with HIV hospitalized with severe pneumonia. In infants with clinical or confirmed TB diagnoses are excluded from entry, as are infants with close TB contacts. At enrollment, stool and nasopharyngeal aspirates are collected for Xpert Ultra testing, and urine for lipopolysaccharide (LAM). TB culture was not performed. Infants enrolled between March 2020-July 2023 were included in this interim analysis.

Results: Laboratory-confirmed TB was present in 23% (114/496) infants; 96% (109/114) presented with >1 Graham clinical criteria and 71% (81/114) with >2. The distribution of patients with specific clinical criteria is shown in Figure 1. Persistent cough was the only criteria with a significant relative risk for laboratory-confirmed TB (1.51 [1.12;2.05], p=0.008). The presence of >1 clinical criteria had a sensitivity of 96%, specificity of 81%, positive predictive value (PPV) of 32.3%, and negative predictive value (NPV) of 80% for laboratory-confirmed TB. The presence of >2 criteria had sensitivity of 71.1%, specificity of 42.7%, PPV of 36.3% and NPV of 76.3%.

Conclusion: Despite excluding the patients most likely to have TB, almost one quarter of infants living with HIV hospitalized with severe pneumonia had Xpert or LAM-confirmed TB after study enrollment, and almost all of them had at least one of the signs/symptoms in the Graham clinical criteria, suggesting that these criteria are valid for this specific patient population.

Influence of Childhood Adversity on Cardiovascular Health in HIV Youth in Uganda

Sahebra Dirajal-Fargo1, Shan Sun1, Christine Karungu2, Joy Gumikiriza-Onoria2, Angel Nanteza3, Nicholas Funderberg1, Victor Musiime4, Grace A. McComsey1, Reuben Robbins5, Jahit Sacarlal1, Nicholas Funderberg1, Robert H Lune Children’s Hospital of Chicago, Chicago, IL, USA, 2Joint Clinical Research Centre, Kampala, Uganda, 3Makerere University College of Health Sciences, Kampala, Uganda, 4The Ohio State University, Columbus, OH, USA, 5Case Western Reserve University, Cleveland, OH, USA

Background: Data suggest that adverse childhood experiences (ACEs) are associated with an increased risk of cardiovascular disease (CVD). However, little data exist on the effect of ACEs and health in children. We examined the relationship between ACEs and CVD risk factors in youth living with perinatally acquired HIV (YPHIV) in Uganda.

Methods: A prospective observational cohort study was performed in 49 YPHIV and 51 HIV- from 2017-2021 at the JCRC in Uganda at baseline and 96 weeks later. All participants were between 10-18 years of age. YPHIVs were on ART with HIV-1 RNA level ≤400 copies/mL. Mean common carotid artery intima-media thickness (IMT), pulse wave velocity (PWV), plasma and cellular markers of systemic inflammation and immune activation were evaluated at baseline and 96 weeks. The Adverse Childhood Experiences-International Questionnaire (ACEs), Patient Health Questionnaire-9 (PHQ-9) and socioeconomic questionnaires were administered, and ACEs sub scored of abuse, neglect and household dysfunction (HHDYS) were calculated. Hierarchical cluster analysis was performed to identify natural clusters of ACEs and socioeconomic factors.

Results: At baseline, median age was 12 years (IQR: 11-14), 52% were female. YPHIV were more likely to have a history of abuse, and higher ACE total scores (p=0.015). Two optimal clusters were derived from ACEs, PHQ-9 mean scores, and socioeconomic variables (Figure 1). Compared to cluster 1, participants in cluster 2 had higher ACEs (p≤0.001 for all), and were more likely to have: HIV (65% vs 42%, p=0.019), higher levels of monocytes and T cell activation (CD14+CD16- and CD14+CD16+ monocytes, CD8 expressing CD38 and HLA DR, p=0.037); higher systolic blood pressure (p=0.040), and higher increases in PWV over 96 weeks (p=0.047). In mixed linear regression models adjusting for HIV status, age, gender, physical activity (met-kcal/hour), mean PHQ-9 score, monocytes, activated CD8 T cells, total ACE score (β=0.10) and HHDYS (β=-0.09) remained associated with a higher change in PWV over 96 weeks.

Conclusion: Findings suggest that ACEs may contribute to CVD risk in YPHIV in Uganda, even after adjusting for factors known to influence cardiovascular health. Early life stress may play an important role on inflammation and cardiovascular health in this setting. Further research is warranted to determine the impact of emotional events on physical outcomes in HIV, whether this is a potentially modifiable risk factor, and how to mitigate long-term consequences.

Changes in the Lipidome Are Associated With Immune Activation in Ugandan PHIV

Sahebra Dirajal-Fargo1, Meliça Nikahd2, Kate Alistock3, Manjumith Manubolu4, Victor Musiime5, Grace A. McComsey1, Nicholas Funderberg1, 1Ann & Robert H Lune Children’s Hospital of Chicago, Chicago, IL, USA, 2The Ohio State University, Columbus, OH, USA, 3Joint Clinical Research Centre, Kampala, Uganda, 4Case Western Reserve University, Cleveland, OH, USA, 5Case Western Reserve University, New York, NY, USA

Background: Linkages between sympathetic activation and immune activation have been observed in children and young adults with perinatally acquired HIV (PHIV). However, little is known about potential linkages between the lipidome and putative immune activation markers in children and youth living with PHIV.

Methods: A longitudinal cross-sectional analysis of lipid species and ACEs was performed in 51 PHIV and 30 HIV- from 2017-2021 at the JCRC in Uganda at baseline and 96 weeks. The Adverse Childhood Experiences-International Questionnaire (ACEs) were administered, and ACEs sub scored of abuse, neglect and household dysfunction (HHDYS) were calculated. Hierarchical cluster analysis was performed to identify natural clusters of ACEs and socioeconomic factors.

Results: Age and gender remained associated with a higher change in PWV over 96 weeks (p=0.047). In mixed linear regression models adjusting for HIV status, age, gender, physical activity (met-kcal/hour), mean PHQ-9 score, monocytes, activated CD8 T cells, total ACE score (β=0.10) and HHDYS (β=-0.09) remained associated with a higher change in PWV over 96 weeks.

Conclusion: Findings suggest that ACEs may contribute to CVD risk in YPHIV in Uganda, even after adjusting for factors known to influence cardiovascular health. Early life stress may play an important role on inflammation and cardiovascular health in this setting. Further research is warranted to determine the impact of emotional events on physical outcomes in HIV, whether this is a potentially modifiable risk factor, and how to mitigate long-term consequences.
measured by direct infusion-tandem mass spectrometry from 100 ART-treated PHIV and 98 age- and sex-matched HIV-Ugandan children at baseline and 96 weeks. All participants were between 10–18 yrs of age. PHIVs had HIV-1 RNA level ≤50 c/ml. In addition, plasma markers of systemic inflammation (hsCRP, IL6), monocyte activation (sCD14 and sCD163), gut integrity and translocation (I-FABP and BDG) were measured by ELISA. T cell activation (expression of CD38 and HLA-DR) on CD4+ and CD8+ T cells was measured by flow cytometry. Comparisons of lipid concentrations between groups were evaluated using 2-sample t-test. Spearman correlations were used to assess correlations between changes in lipid concentration and immune activation. Results: Overall, median age (IQR) was 12 years (11–14); 52% were females. In PHIV, median CD4+ cell counts were 988 cells/µL, and 85% had viral load <50 copies/mL throughout the study. Total cholesterol, LDL, and HDL were similar between the groups, however, the concentrations of ceramides, diacylglycerols, free fatty acids, lysophosphatidycholines and phosphatidylcholines, were significantly higher in PHIV (p<0.03). A network figure highlights the associations between changes in lipid species concentrations and inflammatory biomarkers over 96 weeks with a correlation >0.4. Notable trends included the predominant association with increases in unsaturated triacylglycerols with increased activation of CD4+ and CD8+ T cells and in fungal translocation, and with decreases in sCD14 and IFAB. Conclusion: Despite similar basic lipid panels as HIV-, virologically suppressed PHIV on ART have elevated lipid species that are known to be associated with CVD. Our network analysis identified that triacylglycerols with long and unsaturated acyl chains, previously shown to be associated with an increased risk of plagues in adults living with HIV, are associated with immune activation and fungal translocation. Further studies are warranted to determine whether these lipid species may serve as novel biomarkers. The figure, table, or graphic for this abstract has been removed.

**969 Activated Proinflammatory NK Cells Promote Atherogenesis in Adolescents w/ Perinatally Acquired HIV**

Mario J. Alles1, Manjusa G. Gunasena1, Victor Musimne1, Cissy Kitoy2, Banumathi Tamilselvan2, Brian Richardson3, Wendy Ching-Wen Li4, Cheryl Cameron4, Mark Cameron4, Sahera Birkal-Farge3, Nicholas Funderburg3, Hamal P. Liyanage2

1The Ohio State University, Columbus, OH, USA, 2Makerere University, Kampala, Uganda, 3Joint Clinical Research Centre, Kampala, Uganda, 4Case Western Reserve University, Cleveland, OH, USA, 5Northwestern University, Chicago, IL, USA

**Background:** Perinatally acquired HIV (PHIV) and lifelong antiretroviral therapy (ART) may alter the development and function of the immune system. Published literature and our preliminary data suggest reprogramming of innate immune cells may accelerate aging and increase the risk for future end-organ complications, including cardiovascular disease (CVD). Natural killer (NK) cells are a heterogeneous group of innate immune cells with divergent functions; little is known about the role of NK cells in HIV-associated atherogenesis.

**Methods:** In this cross-sectional study, using high dimensional flow cytometry, plasma biomarker profiling, and transcriptomics, we compared immune signatures in cryopreserved peripheral blood mononuclear cells and cardiovascular biomarkers in Ugandan adolescents with PHIV on ART (n=18), and age/sex-matched HIV-unexposed and uninfected adolescents (n=20). Statistical comparisons employed the Mann-Whitney U test, with significance at p<0.05. We explored the connection between immune signatures and plasma biomarkers using the Pearson correlation coefficient.

**Results:** The median age was 14 years, and 50% were females and all PHIVs were virally suppressed (HIV-1 RNA <50 c/ml). Among PHIVs, markers of activation (CD69, HLA-DR, NKp44), maturation and memory (CD57), and migration to inflamed tissue (CXCR3) were elevated in most NK subsets (based on CD56 and CD16 expression) compared to levels in HIV (p<0.05 for all). Oxidized LDL (ox-LDL) levels were significantly lower in the plasma of PHIVs (p<0.05). Further, negative correlations were found between most of the activated NK subsets expressing chemokine receptor CCR5 and plasma ox-LDL among PHIVs (p<0.05 for all). Our in vitro studies confirmed increased uptake of ox-LDL by macrophages in the presence of activated NK cells (p<0.05). Bulk-RNA sequencing data revealed differential expression of genes associated with immune cell migration, cholesterol uptake into tissue, vascular remodeling, and enrichment of pathways associated with NK activation and epigenetic regulation in the PHIV group (p<0.05).

**Conclusion:** Our data demonstrate, for the first time, increased expression of several activated, mature NK subsets to the potential to home to vascular tissue and influence increased uptake of plasma ox-LDL into vessel wall macrophages and initiate atherogenesis in adolescents with PHIV. We are currently performing mechanistic and longitudinal studies to confirm these findings.

**970 DNA Methylation Signatures of Inflammation in Youth Living With Perinatally-Acquired HIV**

Stephanie Shiu1, Sean Brummell2, Jasmine Douglass1, Francesca Zumpano1, Michael J. Corley3, Jennifer Jao3, Murli Purswani4, Kunjal Patel5, Carmen Marist1, for the Pediatric HIV/AIDS Cohort Study (PHACS)

1Rutgers University, Piscataway, NJ, USA, 2Harvard TH Chan School of Public Health, Boston, MA, USA, 3Weil Cornell Medicine, New York, NY, USA, 4Northwestern University, Chicago, IL, USA, 5Bronx-Lebanon Hospital Center, Bronx, NY, USA

**Background:** Epigenetic modifications may highlight mechanisms through which HIV and antiretroviral therapy (ART) exposure during critical developmental periods affect biological pathways and disease risk. We examined if perinatally-acquired HIV is associated with genome-wide alterations in the DNA methylome by comparing youth living with perinatal HIV (YPHIV) and youth who are perinatally HIV-exposed uninfected (YPUE) at two timepoints. Among YPHIV, we examined associations between viral load (VL), CD4 count, and markers of inflammation with genome-wide alterations in the DNA methylome.

**Methods:** 32 YPHIV and 7 YPUE with peripheral blood mononuclear cell samples collected at two timepoints ≥3 years apart were selected from the US-based PHACS Adolescent Master Protocol. DNA methylation was assayed using the Illumina MethylationEPIC (850K) array. Using the limma package, we tested for differentially methylated (DM) CpG sites (FOR p-value <0.05 and ≥5% difference in methylation) between groups at each timepoint. 15 targeted genes identified from prior work (PMID: 31324826) were also assessed for DM CpG sites. Among YPHIV, genome-wide associations of cumulative VL, cumulative CD4, C-reactive protein [CRP], and Interleukin-6 [IL-6] with DNA methylation were adjusted for ART type and age at ART initiation.

**Results:** Overall, median age was 11 and 17 years at timepoints 1 and 2, respectively. Groups were balanced by sex (51% male) and race/ethnicity (64% non-Hispanic Black). Genome-wide, there were no DM sites comparing YPHIV to YPUE at either timepoint. For targeted genes, 1 CpG site on SPERT and 6 CpG sites on PSMB9 were DM at timepoint 1 comparing YPHIV to YPUE. At timepoint 2, 10 CpG sites on PSMB9 and 4 CpG sites on EBF4 were DM comparing YPHIV to YPUE. Among YPHIV, cumulative VL or CD4 count were not associated with any CpG sites. CRP was associated with 27 CpG sites (16 genes) at timepoint 1 and 19 CpG sites (15 genes) at timepoint 2, with 11 genes overlapping timepoints (Fig 1). IL-6 was associated with 6 CpG sites (4 genes) at timepoint 1 and 2 CpG sites (2 genes) at timepoint 2, with 2 genes overlapping timepoints (Fig 1).

**Conclusion:** Associations between markers of inflammation and epigenetic signatures among genes involved in blood pressure regulation, chronic kidney disease, and fatty acid processing were detected in YPHIV at two timepoints in childhood/adolescence. These genes may provide insight into inflammatory pathways contributing to HIV-associated chronic comorbidities among YPHIV.

**971 Young CPHIV Have Low Proinsulin to C-Peptide Ratios That Inversely Correlate With T-Cell Exhaustion**

Wei Li1, Emily Sims Emily Sims1, Mussa Mwamzuka2, Fatma Marshed3, Aabid Ahmed2, Alka Khaitan1

1Indiana University School of Medicine, Indianapolis, IN, USA, 2Boma Hospital, Mombasa, Kenya

**Background:** Persons with HIV have an increased risk of developing diabetes mellitus. The ratio of immature (Pro-insulin) relative to mature (C-peptide) insulin products (PI:C) in circulation serves as a biomarker of pancreatic β cell stress and predicts progression to type 1 or type 2 diabetes. In adults with HIV
lower PI:C was observed in untreated persons compared to those on treatment or without HIV, suggesting immune dysregulation may preserve β cell function. Children with perinatal HIV (CPHIV) have higher rates of insulin resistance compared with healthy children, but whether β cell dysfunction contributes is unknown. We investigated PI:C ratios in CPHIV and their correlations with clinical and immune activation and exhaustion markers.

**Methods:** We quantified plasma levels of proinsulin (TECO intact proinsulin ELISA) and C-peptide (TOSOH immunoassay) in 200 Kenyan children who were HIV unexposed (HU) and CPHIV who were treatment naïve (ART-) or virally suppressed on treatment (ART+) aged 0-5 years (“0-5y” n=28 ART-, 21 ART+, 36 HU) and 5-20 years (“5-20y” n=41 ART-, 28 ART+, 46 HU). We calculated PI:C and assessed correlations with HIV viral load, CD4%, CD4:CD8, monocyte (CD14+ sCD163), T cell (CD38+HLA-DR+), and systemic (CRP, IL-6) activation markers and immune checkpoints (ICPs: PD-1, CD160, TIM3) on memory T cells. Mann-Whitney and Spearman’s correlations were performed on GraphPad Prism.

**Results:** Compared to HU, 0-5y ART- had lower proinsulin levels (p=0.005) and PI:C (p=0.004), whereas ART+ trended toward lower PI:C (p=0.06). In 5-20y, both ART- and ART+ had a higher C-peptide level (p=0.006 and p=0.04), and ART+ had lower PI:C (p=0.04). In both age groups in CPHIV, PI:C did not correlate with age, viral load, CD4% levels, or activation markers. However, in 0-5y CPHIV PI:C inversely correlated with PD-1 (p=0.05, r=-0.46) and TIM3 (p=0.01, r=-0.40) on memory CD4 T cells as well as PD-1 (p=0.03, r=-0.51) and CD160 (p=0.04, r=-0.32) on memory CD8 T cells. There were no significant correlations between PI:C and ICPs in older CPHIV.

**Conclusion:** CPHIV had a lower PI:C compared with HU, stemming from lower proinsulin in younger CPHIV and higher C-peptide in older CPHIV. Clinical markers of HIV disease progression and inflammation were not associated with β cell stress in CPHIV. T cell exhaustion correlated with PI:C in younger CPHIV. Overall, our data show no evidence of β cell dysfunction in CPHIV and suggest ICPs may play a protective role against β cell stress in young CPHIV.

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972 Metabolic Signature in Youth With Perinatally Acquired HIV and Non-Alcoholic Fatty Liver Disease

**Silvia Chafino**, Laura Taracín-Díez, Jara Hurtado-Gallego, Sonia Alcolea, Antonio Oliver, María Luisa Navarro, Salvador Fernández-Arroyo, Consuelo Viladés, María Luisa Montes, Francesc Vidal, Joaquín Peraire, Anna Rull, Tala Sainz, Hospital Universitario de Tarragona Joan XXIII, Tarragona, Spain, Hospital General Universitario Gregorio Marañón, Madrid, Spain, La Paz University Hospital, Madrid, Spain, Hospital Universitario Álava, Vitoria University, Tarragona, Spain, Hospital La Paz Institute for Health Research, Madrid, Spain

**Background:** Non-alcoholic fatty liver disease (NAFLD) is characterized by accumulated fat producing hepatocellular inflammation and injury. Persistent immune activation and chronic inflammation in response to HIV infection may be factors underlying the development of NAFLD which is recognized as a cause of liver disease although currently diagnosis and management are a challenge. Specifically, metabolic changes and biomarkers associated with this pathology are poorly understood currently in perinatally acquired HIV (PHIV).

**Methods:** Plasmatic lipidomic and bile acids were analysed by LC-QTOF and HPLC-MS/MS in the study cohort consisting of 29 youth living with HIV under antiretroviral treatment (ART) for at least 2 years, aged 8-16 years, were recruited from HIV clinics as were children of similar ages without HIV (CWH) from nearby schools in Harare, Zimbabwe. Dual X-ray absorptiometry was performed at baseline and at 12months quantifying height-adjusted total-body less-head bone mineral content for lean mass (TBLH-BMC) and size-adjusted lumbar spine bone mineral apparent density (LS-BMAD) Z-scores. Change in Z-scores accounted for follow-up time. Baseline plasma levels for CRP, sCD14, TNFα, IL-6, IL-17a, IL-18, IL-10 and IFNα were measured using Lumienx. Bone outcome measurements were univariately plotted with age, with linear regression used to compare longitudinal changes in bone density by HIV status, adjusting for age, pubertal stage and baseline bone density. Principal components analysis was used to group inflammatory biomarkers. Linear models were used to compare these components by HIV status adjusting for age and pubertal stage.

**Results:** There were 275 CWH (mean±SD 12.6±2.5 years old, 134[49.6%] girls) and 283 CWH (12.7±2.5 years old, 149[50.4%] girls) at baseline. No deaths were reported, but 19% were lost to follow-up. ART regimens included either a non-nucleoside reverse-transcriptase or protease inhibitor; 211 (70%) and 89 (29%) respectively. HIV was associated with impaired gains in bone density, particularly among males (Figure 1). While females with and without HIV had similar bone density gains, males living with HIV gained less LS-BMAD (adjusted mean difference[95% CI] -0.14 [-0.25 to -0.02], p=0.02) and TBLH-BMC (-0.19[-0.33 to -0.04], p<0.015) compared to males without HIV. Living with HIV was associated with higher levels of a component representing IL-18, CRP, sCD14 and TNFα for females (coefficient[95% CI] 0.63[0.27 to 0.98], p<0.001) and males (0.80[0.45 to 1.15], p<0.001).

**Conclusion:** Children living with HIV on ART have impaired bone density accrual and increased inflammation. Further investigation is ongoing into the effect of higher inflammation on peak bone mass among this population.
974 WITHDRAWN

975 Association Between HIV and Cytomegalovirus and Neuropsychological Outcomes Among Children With HIV

Jillian Neary1, Daisy Chebet1, Sarah Benki-Nugent1, Hellen Moraa2, Noah Cassidy3, Carolyn Fish1, Barbara Richardson1, Irene Njuguna4, Agnes Langat2, Evelyn Ngugi1, Dara Lehman3, Jennifer Skyler1, Dalton C. Wamalwa1, Grace John-Stewart1

1University of Washington, Seattle, WA, USA, 2University of Nairobi, Nairobi, Kenya, 3Fred Hutchinson Cancer Center, Seattle, WA, USA, 4Kenyatta National Hospital, Nairobi, Kenya

Background: Children with HIV may experience adverse neuropsychological outcomes despite antiretroviral treatment (ART). Uncontrolled cytomegalovirus (CMV) is common in children with HIV. Among children on ART, we examined the influences of early CMV DNA, HIV DNA, and viral load (VL) on neurocognition.

Methods: Children who initiated ART before 12 months of age were enrolled from 2007-2010 in Nairobi, Kenya. Blood was collected at enrollment and every 6 months thereafter. Neuropsychological assessments were conducted when children were a median age of 7 years. Primary outcomes included cognitive ability measured by the Kaufman Assessment Battery for Children 2nd Edition (KABC), executive function measured by Behavior Rating Inventory of Executive Functioning (BRIEF), motor measured by Bruinick’s-Oseretsky Test of Motor Proficiency 2nd Edition Brief Form (BOT), and attention measured by Visual Test of Variables of Attention (TOVA). Secondary outcomes included short-term memory, visual-spatial, learning, non-verbal, and delayed memory from the KABC; behavior regulation and metacognition from the BRIEF; and processing speed from the TOVA. Generalized linear models were used to determine associations between HIV VL (pre-ART and cumulative), peak total and intact HIV DNA (by 12 months of age), peak CMV DNA (by 24 months of age) and neuropsychological outcomes.

Results: Overall, 39 children completed neuropsychological assessments. Median age at ART initiation was 4.6 months. In adjusted models, higher peak CMV viremia by 24 months of age was associated with lower cognitive ability and motor z-scores. Higher pre-ART HIV VL, total HIV DNA, and intact HIV DNA were associated with lower executive function z-scores. Higher HIV DNA levels also were associated with higher motor z-scores and higher intact HIV DNA with higher attention z-scores. Among secondary outcomes, higher intact HIV DNA levels were associated with lower behavior regulation z-scores and higher pre-ART VL was associated with lower nonverbal and metacognition z-scores.

Conclusion: Pre-ART VL, early post-ART total and intact HIV DNA, and CMV DNA in infancy predicted neuropsychological scores in childhood. These findings suggest long-term benefits of early HIV viral suppression, reservoir containment, and CMV control on neurocognition.

976 Regional Brain Volume as Predictor of Cognitive and Mental Health Outcomes in Youth Living With HIV

Sedthapong Chunamchai1, Anantaporn Senna1, Akarin Hiransuthikul1, Phillip Chan1, Robert Paul2, Somchai Sriplienchan2, Thanyawee Puthanakit3, Seraea Spudich1, Chaipat Chunharas1

1King Chulalongkorn Memorial Hospital, Bangkok, Thailand, 2Chulalongkorn University, Bangkok, Thailand, 3Yale University, New Haven, CT, USA, 4University of Missouri St Louis, MO, USA, 5SEARCH, Bangkok, Thailand

Background: Youth living with HIV face a risk of developing cognitive, mental health, and behavioral challenges as they progress into adulthood. However, the specific contributions of biological, neurological, and social factors to these adverse outcomes remain poorly understood. This study seeks to examine factors that can predict resilience at both baseline and after a 2-year period.

Methods: The RESILIENCE study is a 2-year cohort investigation that enrolled youth with perinatal HIV infection and controls. We collected data on various factors, including biological (age, HIV serostatus, viral load), neurological (regional brain volumes), and social (family and financial status). Resilient outcomes were assessed through cognitive function (intelligence, executive function, visuomotor, and memory), mental health (the symptom checklist SCL-90), risk-taking behavior (the behavioral health outcome questionnaire ACASI), and behavioral issues (the child behavior checklist). Multivariable logistic regression analysis with nested model comparisons was employed to identify predictors for each outcome.

Results: The study comprised 30 youth living with HIV and 62 controls, with a median (IQR) age of 15 (13-16) years. At baseline, regional brain volumes emerged as the primary predictor of resilience for cognitive (AUC = 0.65, p < 0.001) and mental health outcomes (AUC = 0.74, p < 0.001). For resilience in risk-taking behavior, a combination of all biological and social factors proved to be the most effective predictor (AUC = 0.70, p < 0.001), while resilience in behavioral issues was best predicted by a combination of regional brain volumes and social factors (AUC = 0.64, p < 0.001). At week 96, regional brain volumes alone were the strongest predictors of cognitive problems (AUC = 0.71, p < 0.001), mental health problems (AUC = 0.74, p < 0.001), and risk-taking behavior (AUC = 0.66, p < 0.001). In contrast, resilience in behavioral issues was predicted by a combination of regional brain volumes and biological factors (AUC = 0.75, p < 0.001).

Conclusion: Our findings reveal distinct predictors associated with resilience to various adverse outcomes in youth living with HIV. Cognitive and mental health outcomes are closely linked to regional brain volume, while behavioral issues and risk-taking behaviors are more strongly associated with a combination of biological and social factors. Recognizing these distinctions could enable targeted identification, monitoring, and intervention strategies for populations at special risk.
Neurocognitive Performance in Adolescents Living With HIV in Zimbabwe

Nyasha V. Dzavakwa,1 Annalie Shears,2 Nicol Redzo,3 Tisiti Bandason,4 Hilda A. Majuru,5 Joseph Piper,6 Victoria Simms,7 Rashida A. Ferrand,8 for the VITALITY Mind Team

Background: Neurocognitive impairment in children and adolescents is complex and multifactorial. This study aimed to characterise the extent and nature of cognitive impairment in adolescents living with HIV (ALWH) in Harare, Zimbabwe and describe interactions between HIV, cognition and stunting.

Methods: In this cross-sectional study, ALWH aged 11–19 years, who established on ART for at least 6 months, were recruited from a public sector HIV clinic. An age-matched HIV negative (HIV-) comparison group was recruited from the same catchment area. Neurocognitive function was evaluated using the Kaufman Assessment Battery for Children 2nd Edition (KABC-II). Anthropometry measurements alongside questionnaires assessing socioeconomic status (SES), school performance and food security were completed. SES was assessed using household asset ownership questions.

Results: 503 participants (251 ALWH, 252 HIV-) were recruited from September 2022 to June 2023 and completed a KABC-II. ALWH median age 16 years, 45% male. HIV negative group median age 15 years, 50.4% male. Most participants aged 11–16 years were in education (95.3% ALWH, 91.2% HIV-). Among those in school, 38.0% of ALWH vs 17.9% of HIV negative participants were below expected school grade for age (p = 0.001). 32% (n = 80) of ALWH were stunted compared to 10.8% (n = 27) of HIV-. More ALWH than the HIV-negative group were in the poorest SES group (29.1% vs 17.1%, p < 0.001) and experienced food insecurity (23.6% vs 13.1%, p = 0.017). Adjusting for age, sex and SES, ALWH scored lower than HIV negative peers on KABC Mental Processing Index (MPI) -3.42 [95%CI -5.33, -1.51] and across all KABC subdomains (Sequential -2.49 [95%CI -4.68, -0.31], Simultaneous -3.83 [95%CI -6.15, 1.51], Learning -1.37 [95%CI -3.42, -0.31], Planning -5.47 [95%CI -7.93, -3.55]). There was evidence of interaction of lower KABC MPI with stunting in ALWH (-4.71 [95%CI -9.62, 0.20]). There was no association between stunting and KABC MPI score in HIV negative adolescents.

Conclusion: Cognitive function in ALWH was impaired across all domains, the effect was magnified in stunted individuals. ALWH faced a multitude of adverse childhood experiences including food insecurity and poverty which may have impacted on their cognitive and physical development. Future longitudinal studies are required to evaluate the impact of nutritional interventions on growth and cognition in ALWH.

Development of Domain-General Cognition in Adolescents With HIV Is Slower Than Healthy Individuals

Anantaporn Sena1,6, Suthapong Chumnahchai2, Akarin Hirunthukul3, Philipp Chan4, Robert Paul5, Somchai Sriplienchan1,7, Thanyawee Puthanakit1,8, Serena Spudich9, Chaipat Chumnaraset,10

1Chulalongkorn University, Bangkok, Thailand, 2King Chulalongkorn Memorial Hospital, Bangkok, Thailand, 3Yale University, New Haven, CT, USA, 4University of Missouri St Louis, St Louis, MO, USA, 5Queen Mary University of London, London, United Kingdom, 6London School of Hygiene & Tropical Medicine, London, United Kingdom

Background: While neuropsychological tests are designed to test specific cognitive abilities, a person who performs well in one task tends to perform well in others (“general cognitive ability” or g-factor). Previous studies showed that g-factor is associated with higher functional connectivity measuring by resting state fMRI. If perinatal HIV exposure can negatively affect white matter integrity, it’s possible that the development of g-factor in these individuals might differ from individuals with no exposure. Here, we studied domain-general cognitive ability and how it differed between older and younger adolescents with different HIV statuses.

Methods: Data from the RESILIENCE study, a cohort conducted from 2015–2019, were analyzed to assess the correlation among 17 neuropsychological tests subscores across 6 cognitive domains. Participants were grouped by perinatal HIV status: HIV-exposed infected children group (HIV), HIV-exposed uninfected children group (HEU), and HIV-unexposed uninfected children group (HUU); and age: early adolescents (10–13 years) and middle adolescents (14–18) years. The number of moderate-to-strong correlations (Spearman’s correlation coefficient ≥ 0.4) between cognitive tests were used as a marker of domain-general cognitive ability. We used permutation methods for the test statistic. Connection strength within and between domains was also explored.

Results: There were 96 HIV, 80 HEU, and 98 HUU in the early adolescent group and 105 HIV, 51 HEU, and 46 HUU in the middle adolescent group with matched genders. At the baseline, we found no difference in the number of connections. However, the HUU group showed a significantly greater increase in connections across age ranges (difference = 34) compared to the HIV (difference = 9, p < 0.05) and HEU (difference = 5, p < 0.001) groups. There was no difference in the increase of connections between the HIV and HEU groups. Additionally, only the HUU group had a significant decrease within-domain connection strength across age groups (average coefficient difference = 0.17, p < 0.001).

Conclusion: Our study reveals a slower progression of general cognitive ability among individuals exposed to HIV. This observation aligns with the well-documented impact of HIV on white matter integrity and extends our comprehension of cognitive development within the HIV-exposed population. Our future research will involve a direct exploration of functional connectivity and its relationship with the g-factor.

Adolescent Outcomes Among Young People With Perinatal HIV Infection and Exposure in the United States

Elaine J. Abrams1, Reuben Robbins1, Afifa Ahmed,2 Curtis Dozelsa3, Luke Kluiza1, Ohemaa Poku1, Michael T. Yin1, Andrew Wiznia1, Claude Mellins4

1Columbia University, New York, NY, USA; 2Jacobi Medical Center, New York, NY, USA

Background: Most young people with perinatal HIV infection (YPPHIV) and with perinatal HIV exposure but who are uninfected (YPPEU) born in the United States are from vulnerable, under-resourced, marginalized communities and are now entering adulthood. Yet, little is known about their adult outcomes (i.e., medical, behavioral, psychiatric, substance use [SU], neurocognitive, and milestone achievement [e.g., employment, school, offspring]).

Methods: CASAH is a New York City-based longitudinal behavioral health cohort study of YPPHIV and YPPEU that began in 2003; data are presented from visits in 2019–23. Psychiatric and SU disorders were assessed using the young adult version of the Diagnostic Interview Schedule for Children, and cognitive functioning with NeuroScreen.

Results: Among 187 participants (124 YPPHIV; 63 YPPEU), mean age was 27.8 years; 60% female; 64% Black, 47% Latino. Among YPPHIV: median CD4+ = 450 cells/mm3; 64% had viral load < 200 copies/ml; 57% received 2NRTI+INSTI or bP/2I; 24% 2NRTI+INSTI or bP/NRTI. Most participants were never married (94%) and currently sexually active (73%); over half in both groups reported condomless sex in past 3 months. Over 50% of females and 42% of males reported pregnancy in self or partner, with no group differences. Overall, 27% met criteria for a non-SU psychiatric disorder (14% depression, 16% anxiety); 32% met criteria for a SU disorder (primarily alcohol and/or marijuana). YPPHIV performed worse on cognitive tests with 20% (vs 4% YPPEU, p = 0.01) having global test performance 2.5SDs below the sample mean. Overall, 78% completed high school or GED; 50% were in school or currently employed with higher rates in YPPHIV vs YPPHIV (65% vs 43%, p = 0.004). YPPHIV were more likely than YPPEU to receive housing assistance (73% vs 53% p = 0.007), public assistance (29% vs 8%, p = 0.001) and food stamps (68% vs 29%, p < 0.001). Homelessness history was higher in YPPHIV (46% vs 26% YPPEU, p = 0.009); 18% of participants reported incarceration history with no HIV-status differences.
980 High Burden of HIV-Related Disease Among Adults With Perinatally-Acquired HIV in Argentina

Violeta Z. Ortíz1, Julian Vega1, Maria L. Santos1, Solange Arazì Gaillaud1, José A. Barletta1, Maria J. Rolón1
1Hospital Juan A. Fernandez, Buenos Aires, Argentina, 2Hospital Juan P. Garrahan, Buenos Aires, Argentina

Background: Limited data is available regarding population size and burden of HIV-related disease among adults living with perinatally-acquired HIV (pHIV) in Latin America. This study is aimed at describing HIV-related burden of disease in a cohort of adults living with pHIV from Buenos Aires, Argentina.

Methods: This is a retrospective cohort study. People living with pHIV aged >16 and linked to care in an HIV referral clinic in Buenos Aires, Argentina between Oct-2008 and Sep-2023 were included. Data was collected from clinical records and epidemiological surveillance systems. Clinical status was classified as per WHO HIV staging system, and advanced HIV disease was defined as WHO stages 3-4. Ethics approval was obtained as appropriate.

Results: A total of 170 adults (60% females) with pHIV were included in the analyses. Median age at baseline was 19 years and median individual follow-up was 5.7 years (Q1-Q3 3.6-9.5). Prevalence of clinically advanced HIV disease was 47% (79/170); 34/79 participants presented 1, 36/79 presented 2-5 and 9/79 presented >5 WHO stage 3-4 events. Graphic 1 shows frequency of incident WHO stage 3-4 events (1a) and proportion of undetectable viral loads and CD4 counts ≥200 cells/μl, per participant (1b) [data available for 161 and 165 individuals, respectively]. There were 133 HIV-related hospital admissions in 49 participants. Global mortality was 11% (18/170) and median age at the time of death was 23.5 years (Q1-Q3 21.2-27). The majority (11/18) of the deaths were HIV-related and one third (6/18) occurred within the first year after transition to adult care.

Conclusion: Adults living with pHIV have high HIV-related morbimortality and HIV-related complications are the leading cause of death in our cohort. To our knowledge, this is the largest description of a cohort of adults living with pHIV in Latin America. Further research is needed to complete characterization of this population in order to design and implement differentiated service models that contemplate their singularity.

Graph 1 - Frequency of WHO stage 3-4 events (1a) and proportion of undetectable viral loads and CD4 counts ≥200 cells/μl, per participant (1b)

981 No Early Signal That DTG Improves 24-Week Viral Suppression in Infants in Botswana

Maureen Sakis-Moselthi1, Gholahan Ajobola1, Oganne Batlang1, Kenneth Maswabi1, Molly Pretorius Holme2, Kathleen M. Powis1,3,4,5, Lipontšo Motaboli1,2,3,4,5, Motlatsi Letsika2,3,4,5, Lipontšo Motaboli1,2,3,4,5, Niklaus D. Labhardt1,2,3,4,5, Michael D. Hughes1,2,3,4,5, Roger Shapiro1,2,3,4,5, Kenneth Maswabi1,2,3,4,5, Akash Devendra1,2,3,4,5, Maurus Kohen1,2,3,4,5, Motlatsi Letsika1,2,3,4,5, Hape Khooa1,2,3,4,5, Lipontšo Motaboli1,2,3,4,5, Malebanye Leratho1,2,3,4,5, Nadine Tschumi1,2,3,4,5, Kenneth Maswabi1,2,3,4,5, Lipontšo Motaboli1,2,3,4,5, Lipontšo Motaboli1,2,3,4,5, Akash Devendra1,2,3,4,5, B satisfied

1 Baylor College of Medicine Children’s Foundation, Maseru, Lesotho, 2University Hospital Basel, Basel, Switzerland, 3SolidarMed, Maseru, Lesotho

Background: Children and adolescents with HIV experience high rates of treatment failure. Antiretroviral therapy (ART) containing dolutegravir has recently been rolled out across much of Africa and has several potential benefits over previously preferred ART regimens, though long-term real-world data in pediatric populations are lacking. Here, we report treatment outcomes among children and adolescents in Lesotho, southern Africa, who transitioned from non-nucleoside reverse transcriptase inhibitor (NNRTI-) to dolutegravir-based ART through two years’ follow-up.

Methods: Data were derived from two open cohort studies in Lesotho (Baylor College of Medicine Children’s Foundation Lesotho and Viral Load Cohort North-East Lesotho). Children and adolescents aged <18 years who transitioned from NNRTI- (efavirenz or nevirapine) to dolutegravir-based ART ≥18 months before data closure were included. We report viral load (VL) results <12 months before, 12 (window: 6-17) months after, and 24 (window: 18-29) months after transition to dolutegravir. Associations of demographic and clinical factors with 24-month viremia were assessed through multivariable logistic regression.

Results: Among 2121 children and adolescents included, 1099 (51.8%) were female. At transition to dolutegravir, median age was 14.0 years (interquartile range [IQR] 11.5-15.8), median time taking ART was 7.6 years (IQR 4.4-10.6), and most participants had been taking an efavirenz-based regimen (1433/2121 [67.6%]). Participants were followed up over a median of 2.6 years (IQR 2.3-3.2). A VL was available for 1971/2121 (92.8%) <12 months before, 2006/2121 (94.6%) 12 months after, and 1887/2121 (89.0%) 24 months after transition to dolutegravir. Among those with a VL result at the respective time points, viral suppression to <50 copies/ml was achieved by 1633/1971 (82.9%) <12 months
before, 1840/2006 (91.7%) 12 months after, and 1708/1887 (90.5%) 24 months after transition to dolutegravir. The Figure shows VL dynamics for participants with VL data at all time points. Pre-transition viremia was associated with viremia at 24 months, though 227/272 (83.5%) and 232/272 (83.5%) participants with pre-transition viremia had achieved resuppression to <50 copies/mL at 12- and 24 months after transition to dolutegravir, respectively.

**Conclusion:** Rates of viremia dropped after transition to dolutegravir. However, further progress is needed to reach global targets in children and adolescents.

**Figure:** Viral load (VL) dynamics among participants with an available VL: 412 months before, 12 months after, and 24 months after transition to dolutegravir (n=1738). No shade: VL category at respective time point; flow shade: VL category before transition.

### 983 Longitudinal Viral Outcomes in Kenyan Youth With HIV Switching to Dolutegravir-Based Therapy

**Vlad Novitsky**¹, Winstone Nyandiko¹, Allison DeLong¹, Edwin Sang¹, Joel Hague¹, Ashley Chory¹, Josephine Aluoch¹, Eshyne Jekembo¹, Millicent Odido¹, Joseph Hogan¹, Rachel Vreeman², Rami Kantor³

¹Brown University, Providence, RI, USA; ²Moi University, Eldoret, Kenya; ³Academic Model Providing Access to Healthcare, Eldoret, Kenya, *Academic Model Providing Access to Healthcare, Eldoret, Kenya, ³University School of Medicine at Mt Sinai, New York, NY, USA

**Background:** Dolutegravir (DTG)-based antiretroviral therapy (ART) is now available in USAID-supported PEPFAR countries. Treatment disengagement is linked to increases in viral load and onward HIV transmission. To better understand continuity of treatment, we analyzed differences in proportions were estimated using Fisher’s exact test.

**Methods:** In a well-characterized longitudinal cohort (since 2010) of perinatally infected CAWH cared for at the Academic Model Providing Access to Health care (AMPATH) in western Kenya, we determined viral outcomes, and compared outcomes between CAWH with and without provider-initiated switch to DTG-based regimens. During the 18-month follow up, viral load (VL) was tested at 6-month intervals and drug resistance genotyping was performed for VL>1000 copies/mL, and interpreted with Stanford HIV Drug Resistance Database tools. Differences in proportions were estimated using Fisher’s exact test.

**Results:** Of 390 CAWH followed for 18 months between July 2020-February 2023 (median age 19 years; 48% female; median 13 years on ART), 83% switched to DTG-based regimens and 17% remained on older regimens (2% NNRTI- and 98% PI-based). Among 324 CAWH on DTG-based regimens at 18 months, 293 (90%) were suppressed at that timepoint, 103 (32%) had VL>40 copies/mL, and 64 (20%) had VL>1000 copies/mL at any follow up time point. Among 66 CAWH on non-DTG-based regimens, 47 (71%) were suppressed at 18 months (p<0.005), 37 (56%) had VL>40 copies/mL, and 30 (45%) had VL>1000 copies/mL at any follow up time point. Of 186 patient-visits with VL>1,000 copies/mL in at least one timepoint during follow up, 141 sequences were available, including 69 from 52/324 (16%) CAWH on DTG-based regimens and 72 from 37/66 (56%) CAWH on non-DTG-based regimens. Any drug resistance was present in 58% of those on DTG-based, and in 86% of those on non-DTG-based regimens (p<0.01), including major PI drug resistance mutations (DRMs) in 2% vs. 16% (p<0.02), NRTI DRMs in 27% vs. 68% (p<0.001) and NNRTI DRMs in 46% vs. 78% (p<0.01), respectively. No major INSTI DRMs were detected, with accessory INSTI DRMs identified in 12% vs. 11% (p-value not significant).

**Conclusion:** Longitudinal data in Kenyan CAWH support switching to DTG-based ART to improve viral suppression and prevent resistance accumulation. Lack of major INSTI DRMs during this short follow up is encouraging, yet cumulative viral failures, including low level viremia, mandate close monitoring of adherence, VL and resistance in this vulnerable population.

### 984 Global Transition to Dolutegravir-Based ART in Children and Adolescents 0-19 Years Living With HIV

**Sophie Desmonde**¹, Kim Anderson¹, Joyeux Bwami¹, Du tuan Quy¹, Winstone Nyandiko¹, Christella Twizere¹, Marco T. Luque¹, Renee De Waal¹, Vohith Khol², Patricia Lele³, Vanessa Roszier⁴, Frankie Odihambo⁵, Valenarie Leroy⁶, for IeDEA

¹Institut National de la Santé et de la Recherche Médicale, Toulouse, France; ²University of Cape Town, Cape Town, South Africa; ³Children’s Hospital, Ho Chi Minh City, Vietnam; ⁴Academic Model Providing Access to Healthcare, Eldoret, Kenya; ⁵Centre National de Référence en Matière de VIH, Bujumbura, Burundi; ⁶Instituto Hondureño de la Seguridad Social, Tegucigalpa, Honduras; ⁷Institutional Core for HIV/AIDS Dermatology and STDs, Phnom Penh, Cambodia; ⁸Kalémbule Lebbe Pediatric Hospital, Kinshasa, Democratic Republic of Congo; ⁹GHESKIO, Port-au-Prince, Haiti; ¹⁰Kenya Medical Research Institute, Kisumu, Kenya

**Background:** Dolutegravir (DTG)-based regimens are recommended as first-line antiretroviral therapy (ART) for all children and adolescents living with HIV (CALHIV). We describe transition to DTG in the multiregional iDEA cohort.

**Methods:** We included all CALHIV enrolled in iDEA sites where DTG was available from 6 regions (Asia-Pacific, Latin America, Central Africa, West Africa, East Africa, Southern Africa). The observation period of the study was from January 2018 through March 2023. Follow-up (FU) for individual patients began on the date at which their site began to use DTG or at ART initiation, whichever occurred later (i.e., baseline); FU ended at DTG initiation or database closure/death/loss to FU (LTFU; no visit >7 months), whichever came first. We computed regional cumulative incidence functions (CIF) for DTG initiation. Associated factors were explored in a Cox proportional hazard model adjusted for sex, age, ART regimen and viral load (VL), and stratified by region, using a calendar scale.

**Results:** Overall, 80% of sites had access to DTG and 61,324 CALHIV were included in our study; 55% were female and 60% were from Southern Africa. At baseline, median age was 12.2 years (IQR: 7.3-16.3), and 37% had VL assessments, of whom 51% were suppressed (<50 copies/mL). Median follow-up was 16.6 months (IQR: 6.0-27.8) during which 711 (1%) CALHIV died and 14,514 (24%) were LTFU. The overall CIF for DTG initiation reached 92% (95%CI: 91.9-93) and was significantly lower in the Asia-Pacific (44%; 95%CI:41-47) compared to other regions (Figure). In adjusted models, CALHIV <5 years (compared with older CALHIV), those with detectable VL (compared to those with undetectable VL), and those on protease inhibitor-based regimens (compared with non-nucleoside-based regimens) were less likely to initiate or transition to DTG. Additionally, we found in all 4 African regions that female CALHIV were significantly less likely to access DTG than their male counterparts. In other regions, sex was not associated with initiation or transition to DTG.

**Conclusion:** We report an unequal transition to DTG in sites where it has been available. Access to pediatric DTG should be scaled up for younger CALHIV. Moreover, our results highlight the need to promote equitable use of DTG regardless of sex and VL. Continued documentation of treatment practices is required to ensure universal and equal access to DTG for all CALHIV.

### 985 Treatment Interruption Patterns Among Young People in USAID-Supported PEPFAR Programs

**Tishina Okegbé**¹, Lana Lee², Nashiva McDavid³, Madeline Schneider⁴

¹United States Agency for International Development, Washington, DC, USA; ²Credence Management Solutions, LLC, Washington, DC, USA

**Background:** Continuity of treatment for people living with HIV is paramount in order to achieve the UNAIDS 95-95-95 targets and reach epidemic control. Treatment disengagement is linked to increases in viral load and onward HIV transmission. To better understand continuity of treatment, we analyzed treatment interruption patterns among children, adolescents, and young adults in USAID-supported PEPFAR countries.

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**Figure:** Estimated cumulative incidence (CIF) of DTG initiation among CALHIV aged 0-19 years in sites where DTG has been available, by region, in iDEA.
Methods: Routinely collected programmatic data from 42 USAID-supported PEPFAR country and regional programs were analyzed for U.S. fiscal year (FY) 2022 Quarter(Q) 3 through FY2023 Q2 (April 2022 - March 2023). We compared trends in the percent and volume of interruptions in treatment (IIT; overall, <3 months on treatment, 3-5 months on treatment, and 6+ months on treatment) among children (0-9 years), adolescents (10-19 years), and young adults (20-29 years). Interruption in treatment is defined as no clinical contact for 28 days after the last expected clinical appointment or medication pick-up date.

Results: Comparing FY22Q3 to FY22Q2, the overall %IIT as well as absolute number of IIT decreased for all age groups: children (2.8% vs 2.6%; 3,613 vs 3,303), adolescents (2.8% vs 2.6%; 8,141 vs 7,853), and young adults (3.5% vs 3.4%; 44,721 vs 37,435); however, a peak in %IIT was observed for all ages in FY23Q1 (3.2%, 3.1% and 4.1%, respectively). Though overall %IIT is highest for young adults in all quarters, in FY22Q4 through FY23Q2 the highest rates of %IIT occurred in adolescents who have been on treatment for <3 months (12.1%, 14.9% and 11.9%) followed by adolescents who have been on treatment for 3-5 months (11.2%, 12.7% and 8.0%). %IIT among those who have been on treatment for 6+ months is greatest for young adults across all four quarters. For all age groups between FY22Q4 and FY23Q2, the absolute number of IIT is largest at 6+ months; however, compared to overall %IIT, higher rates of %IIT are observed within the first three months on treatment (ranging from 2.7 - 4.8 times more often) followed by 3-5 months (ranging from 2.1 - 4.8 times more often).

Conclusion: Overall rates of IIT among children, adolescents, and young adults in USAID-supported PEPFAR programs between FY22Q3 and FY22Q2 remain high. The pattern of markedly high rates of IIT in individuals, particularly adolescents, on treatment <3 months and 3-5 months, highlights the need for targeted interventions and support for new initiators to ensure continuity of treatment.
Emerging Integrase Resistance in the Perinatal Virtual Clinic: The Need for Protease Inhibitors

Caroline Foster, Ayoliola Eni-Eni-Oludum, Angela Bailey, Alasdaire Bamford, Hermione Lyall, Julia Kenny, Leon Levin, Katherine R. Simon, Tiago Milheiro Silva, Neil Tickner, Anna Turkova, Steven Welch, Nicola Mackie, for the Perinatal Virtual Clinic at Imperial College

1Imperial College Healthcare NHS Trust, London, United Kingdom, 2Imperial College London, London, United Kingdom, 3Buckinghamshire Healthcare NHS Trust, Oxford, United Kingdom, 4Great Ormond Street NHS Foundation Trust, London, United Kingdom, 5 Evelina London Children’s Hospital, London, United Kingdom, 6Department of Paediatrics, Royal Children’s Hospital, Melbourne, Australia, 7Baylor College of Medicine Children’s Foundation, Lilenwe, Malawi, 8Centre Hospitalier Universitaire de Limoges Central, Limoges, Portugal, 9U.C. Great Ormond Street Institute of Child Health, London, United Kingdom, 10University Hospitals Birmingham, Birmingham, United Kingdom

Background: The perinatal virtual clinic (PVC); a monthly multidisciplinary forum reviews complex management decisions for children and adolescents living with HIV (CALWHIV) referred from high (HIC) and low/middle income countries (L/MIC). We investigated the prevalence of emergent integrase drug resistance mutations (INSTI-DRMs) necessitating protease inhibitor (PI) based regimens.

Methods: Review of 5 years of PVC referrals; October 2018-September 2023. Demographic data included age, sex, country of residence. Clinical data included: comorbidities, antiretroviral therapy (ART) history, HIV viral load (VL) and CD4 count. Resistance mutations were interpreted using the Stanford HIV Drug Resistance database and CALWHIV with emergent INSTI-DRMs described.

Results: 274 CALWHIV were discussed; 51 (15%) from L/MIC, 106 (31%) with virological failure (VF) of which 96 (91%) had resistance sequences available. 17/96 (18%) had INSTI-DRMs, median (IQR) age 11 (6-14) years, weight 24 (17-49) kg, CD4 430 (77-805) cells/ul, and VL 35,000 (2380-132000) c/ml. 17/96 (18%) had INSTI-DRMs, median (IQR) age 11 (6-14) years, weight 24 (17-49) kg, CD4 430 (77-805) cells/ul, and VL 35,000 (2380-132000) c/ml. Current region; Africa (7), Americas (2), Europe (5) and Western Pacific (2), with (17-49) kg, CD4 430 (77-805) cells/ul, and VL 35,000 (2380-132000) c/ml. 17/96 (18%) had INSTI-DRMs, median (IQR) age 11 (6-14) years, weight 24 (17-49) kg, CD4 430 (77-805) cells/ul, and VL 35,000 (2380-132000) c/ml.

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Conclusion: INSTI resistance is emerging in CALWHIV, most commonly in highly treatment experienced individuals from L/MIC. This highlights the global need for access to DRV/r, TXF and novel classes, including formulations for children.
991 Evaluation of Electronic Peer Navigation to Prevent Engagement Failure for Youth in Kenya
I. Lisa Abuogi1, Lina M. Montoya2, Edwin Ngaya3, Jayne L. Lewis-Kulzer4, Everlyne Nyandieka5, Gladys Onguta1, Lyssa Opondo1, James Nyanga6, Elvid Akama1, Thomas Odony1, Elizabeth Bukusi7, Elvin H. Geng8
1University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 3Kenya Medical Research Institute, Nairobi, Kenya, 4University of California San Francisco, San Francisco, CA, USA, 5Washington University in St. Louis, St Louis, MO, USA

Background: Adolescents and Young Adults with HIV (AYAHIV) require innovative approaches to address lower rates of virologic suppression and HIV care engagement. While peer-based interventions have strong developmental justification, AYA have many competing demands including school, life transitions, and emerging social needs that may limit reach of in-person support services. The ubiquity of mobile phones and social media, however, offers an alternative route for peer engagement and support.

Methods: We randomized AYAHIV to trained virtual peer navigators who delivered structured peer support electronically (by phone) combined with biweekly automated text messages. We enrolled AYAHIV aged 14-24 years at three high volume public facilities in Kisumu County, Kenya between April 2021 and March 2022. Participants were block randomized to standard of care (SOC) or electronic navigations (eNAV) stratified by ages 14-19 and 20-24 years. Electronic navigators were trained youth with HIV. The primary outcome was AYAHIV engagement failure at one year defined as experiencing any of the following within the first year of follow-up: missed clinic visit (at least 14 days late for a scheduled visit) or viral failure (high viral load per national guidelines) or death. Targeted maximum likelihood estimation was used to estimate effect of virtual navigation versus SOC. We adjusted for baseline patient characteristics (e.g., sex, age, WHO stage, alcohol use, school attendance, etc.) to enhance precision.

Results: Of the 579 participants, 285 (49.2%) were randomized to eNAV and 294 (50.8%) to SOC. Among all patients, 403 (69.6%) were female and median age was 20 years (interquartile range 17-23). Treatment assignment was balanced by sex, age, WHO Stage, study site, or school enrollment between arms (Table 1). Overall, 75 (26.3%) AYAHIV experienced and engaged failure in eNAV and 93 (31.6%) in SOC resulting in an estimated risk difference of -5.18% (95% confidence interval -12.54%, 2.19%, p=0.168). The risk difference was also non-significant in unadjusted analysis.

Conclusion: Results from this trial comparing peer navigation and text messaging versus the standard of care demonstrate high levels of engagement failure in AYAHIV that are reduced, but not statistically significantly, through electronic navigation. Peer support to increase treatment success may require more intensive in-person interactions, despite the reach and flexibility provided by electronic approaches.

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992 Effectiveness of the mHealth Intervention, InTSHA, Among Adolescents With HIV in South Africa
Brian C. Zannoni1, Mohrendran Archary2, Thobekile Sibaya3, Casie T. Gethers4, Madeleine Goldstein5, Scarlett Bergman6, Christina Psaros4, Vincent Marcon6, Jessica Haberer5
1Elimn Tree University, Atlanta, GA, USA, 2University of Kwazulu-Natal, Durban, South Africa, 3George Washington University, Washington, DC, USA, 4Massachusetts General Hospital, Boston, MA, USA

Background: Retention in care for adolescents with HIV during transition from pediatric to adult care is more challenging than for younger children or older adults not transitioning. We describe the results of a pilot, type 3 hybrid, randomized clinical trial of a mobile phone-based intervention, InTSHA: Interactive Transition Support for Adolescents with HIV, compared to standard care.

Methods: InTSHA uses encrypted, closed group chats delivered via WhatsApp to provide peer support and improve communication between adolescents, their caregivers, and healthcare providers during transition from pediatric to adult care. We randomized 80 South African adolescents with perinatally-acquired HIV who were aware of their HIV status and aged between 15 to 19 years to receive either the InTSHA intervention or standard care. We measured acceptability (Acceptability Implementation Measure) and feasibility (Feasibility Implementation Measure) in those randomized to InTSHA as primary outcomes. We also measured retention in care (missed clinic visits and/or late pharmacy refills), viral suppression (viral load <200 copies/ml, depression [PHQ-9], transition readiness (HIV Adolescent Readiness for Transition), peer support (Adolescent Social Support Scale), and connection to clinical staff (Working Alliance Inventory) at baseline and 12 months after randomization. We examined the differences from baseline and 12 months in the InTSHA and standard care groups using independent sample t-tests and chi-square tests.

Results: Among adolescents randomized to the InTSHA intervention (n=40) versus standard of care (n=40), we found no difference in 12-month viral suppression rates n=32, 80% and n=34, 85%, respectively (OR 0.7, 95% CI 0.2 – 2.3; p=0.56). All participants were retained in care at one year. Among adolescents randomized to the InTSHA group, acceptability was 80% and feasibility was 78%. Non-significant improvement was seen in scores for depression 0.8 vs 1.47 (p = 0.68), peer support 2.1 vs 1.7 (p =0.19), transition readiness 0.4 vs 0.1 (p =0.35), and connection to clinic 1.3 vs 0.4 (p =0.55) comparing baseline to 12-month responses in InTSHA compared to standard care respectively.

Conclusion: InTSHA is an acceptable and feasible intervention for adolescents with HIV who are transitioning to adult care in South Africa. Although this pilot study did not improve viral suppression or retention in care, potential improvements were seen in depression, peer support, transition readiness, and connection to clinic.

993 Is HIV Outbreak Among Children in Larkana Over? Findings From a Large Test and Treat Initiative
Muhammad S. Jamil1, Muhammad S. Pasha2, Shahida Memoon1, Altaf Ali1, Tanweer Hussain1, Altaf A. Soomro3, Saima Mushtag1, Sikandar Memoon1, Jomnuna Hermez4
1World Health Organization Regional Office for Eastern Mediterranean, Cairo, Egypt, 2World Health Organization Country Office Pakistan, Islamabad, Pakistan, 3HIV Treatment and Support Centre Rotadero, Larkana, Pakistan, 4Bridge Consultants Foundation, Karachi, Pakistan, 5Communicable Disease Control (HIV-AIDS), Karachi, Pakistan

Background: An outbreak of HIV among children was reported in Rotadero (district Larkana, Sindh province, Pakistan) in April 2019. The main sources of transmission were nosocomial namely, unsafe injections and infusions in healthcare settings. In 2022, routine ART registration data suggested ongoing community transmission with more adults being diagnosed than children, but positivity rates were unclear given the lack of testing data. We present the results of a community-based educate, test and treat initiative in Rotadero to understand the status of outbreak nearly five years after it was first reported.

Methods: Door-to-door testing was done in partnership with CDC Sindh and local administration which focused on selected union councils (UCs) of Rotadero (370000 population), the epicentre of 2019 outbreak. Those aged 18 months to 60 years were eligible, while those who self-reported HIV test in the past 6 months or were already on ART were excluded. Thirty mobile teams of one trained male and female mobilizer each offered a single rapid HIV test (Abbott Early Detect) from house to house in pre-defined geographic areas. Those with a reactive result were referred to Rotadero ART centre for confirmation and ART initiation. Information, education and communication materials related to injection safety were displayed in health facilities and in the community.

Results: Between September 6 and 21, 2022, 43877 HIV tests were performed (58% among females). Overall, 75 individuals (0.17%; male: 0.19%, female: 0.16%) had a reactive HIV test. The reactive rate varied by UC, ranging from 0% to 0.27%. Two-thirds (n=49) of all reactive results were among children (18 months-14 years). The reactive rate among children (0.24%) was higher than those 15 years and above (0.11%). Of those with reactive results, 55 (71%) were linked to ART centre as of January 9, 2024 (53 confirmed HIV-positive and initiated ART and 2 persons initiated TB treatment). The mode of transmission
for 43 individuals (78%) was reported to be reuse of contaminated needles or syringes.

**Conclusion:** HIV positivity was comparable to national HIV prevalence (0.2%) suggesting low-level community transmission. Children continue to be at a greater risk of acquiring HIV and reuse of contaminated needles and syringes continue to drive the transmission in this setting. Urgent action is needed to address unsafe injection practices to stop the transmission.

994 Data Informed Stepped Care (DiSC) to Improve HIV Care for Youth With HIV: A Cluster Randomized Trial

Pamela Kohler1, Wenwen Jiang1, Jacinta Badia1, James Kibugi1, Jessica Dyer1, Julie Kadima1, Dorothy Oketch1, Kristin Beima-Sofie1, Sarah Hicks1, Barbra Richardson2, Irene Inwani3, Seema Shah1, Grace John-Stewart4, Kawango Agot2
1University of Washington, Seattle, WA, USA, 2Impact Research and Development Organization, Kisumu, Kenya, 3Kenyatta National Hospital, Nairobi, Kenya, 4Ron & Robert H Lune Children’s Hospital of Chicago, Chicago, IL, USA

**Background:** Adolescents and young adults living with HIV (YLH) may benefit from differentiated care, however providers and policy makers are hesitant to assign YLH to differentiated care due to concerns over poor retention and viral suppression.

**Methods:** This cluster randomized trial tested effectiveness of a multi-component data-informed stepped care intervention that assigned YLH to different intensities of care according to need. YLH at 12 intervention facilities underwent risk assessment and step assignment at each visit; those at lowest risk were eligible for differentiated services including multi-month refill and pharmacy fast-track. At enrollment YLH received a standardized questionnaire to assess baseline characteristics and were followed for 12 months. Electronic medical record data were abstracted for clinic visit and viral load data. The primary trial outcome was proportion of missed visits during 12-month follow-up. Secondary outcomes included loss to follow-up, viral suppression, and differentiated care assignment. Mixed effects regression was conducted, clustered by individual and facility and adjusted for baseline retention and viral suppression during the pre-enrollment period and for any variable that differed by arm at baseline.

**Results:** Between April to July 2022, 1911 YLH ages 10-24 were enrolled (1016 at control and 895 at intervention facilities). Median age was 17, 1102 (57.9%) were female, and 1512 (79.5%) were in school, and were balanced between trial arms. More YLH attended clinic alone at baseline in the intervention arm. Among YLH in intervention arm, 574 (64.6%) were assigned to differentiated care services, 122 (13.7%) to standard care, 100 (11.3%) to mental health and retention counseling, and 92 (10.4%) to intensive case management. YLH at control sites received standard care. Missed visits were not significantly different between intervention (8.5%) and control groups (8.3%) (aRR 1.04, 95%CI: 0.89-1.20). Assignment to fast-track pharmacy visits increased at intervention sites (aRR 1.21, 95%CI: 1.01-1.45). Viral suppression was similar between arms (aRR 0.78, 95%CI: 0.49-1.23).

**Conclusion:** The data-informed stepped care tool resulted in increased assignment of low risk YLH to fast-track visits without additional loss to follow-up or viral non-suppression. Differentiated services were readily implemented in YLH and may align well with school schedules, decrease health system burden, and enable tailored intensive care for YLH with additional needs.

996 The Impact of the COVID-19 Pandemic on Substance Use Disorder Risk Among People With HIV in the US

Jennifer P. Jain1, Nadra E. Lisha2, Carlos Moreira3, David V. Glidden4, Greer Burkholder5, Heidi M. Crane6, Jeffrey Jacobson7, Edward Cachay7, Kenneth H. Mayer8, Sonia Napravnik9, Richard D. Moore7, Mallory Johnson10, Katerina Christopoulos11, Monica Gandhi12, Matthew A. Spinelli13
1University of California San Francisco, San Francisco, CA, USA, 2University of Alabama at Birmingham, Birmingham, AL, USA, 3University of Washington, Seattle, WA, USA, 4Case Western Reserve University, Cleveland, OH, USA, 5University of California San Diego, La Jolla, CA, USA, 6Fenway Health, Boston, MA, USA, 7Columbia University, New York, NY, USA, 8University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 9The Johns Hopkins University, Baltimore, MD, USA

**Background:** The COVID-19 pandemic has disproportionately impacted vulnerable populations who experience syndemic conditions, including HIV and co-occurring substance use disorder (SUD). We examine here whether there was a significant change in moderate or severe SUD risk among people with HIV (PWH) enrolled in a large multisite clinic-based cohort in the US, before and after the COVID-19 shelter in place (SIP) mandate.

**Methods:** Data collected between March 2018 and October 2022, among PWH enrolled in the Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort across eight sites were used for this study. PWH were asked about their use of the following substances: cannabis, cocaine, amphetaamines, inhalants, sedatives, hallucinocogen, or opioids. Moderate or severe SUD risk was defined as having a score of 4 or greater on the validated ASSIST tool. Using interrupted time series (ITS) analyses, we evaluated whether there was a change in moderate or severe SUD risk over time, comparing trends before and after SIP within a mixed-effects logistic regression model. Analyses were adjusted for age, race/ethnicity, gender, study location, and current HIV viral load.
Results: Data from 7,126 participants, including 21,741 SUD assessments, were included. The median age was 51 (IQR 39–58); 47% of the sample identified as Black, 35% White, 13% Hispanic; and 17% were female. Overall, 43% had used marijuana in the last quarter, 34% cocaine, 15% methamphetamine, and 3% opioids. In the ITS analysis, the rate of moderate/severe SUD risk increased markedly during the pandemic with 43% (95% CI=40–46%) compared to 24% (95% CI=22–26%) having moderate/severe SUD risk post-SIP compared to pre-SIP (P<0.001; Figure).

Conclusion: We found a significant increase in moderate/severe SUD risk among PWH in a large multi-site network of HIV clinics throughout the US. This rising prevalence could be related to an increase in depression, anxiety, and social isolation among PWH during the COVID-19 pandemic. Substance use and misuse are often coping mechanisms for poor mental health conditions, and general life stress. Further, service disruptions due to the pandemic and a transition to telehealth may have caused substantial interruptions in substance use and mental health treatment services among PLWH. To address the combined epidemics of substance use and HIV following the COVID pandemic, a renewed investment in integrated substance use treatment and mental health services is vital.

Validation of a Cumulative Substance Use Biomarker Improves Methamphetamine Use Detection in PWH

Ayesha Appa1, Marjan Javanbakht2, Rachel Bolanos3, Hideaki Okochi1, Karen Kuncz1, Alexander Louie1, Matthew A. Spinelli1, Pamela Gorbach1, Monica Gandhi2
1University of California San Francisco, San Francisco, CA, USA; 2University of California Los Angeles, Los Angeles, CA, USA

Background: Methamphetamine (MA) use is a predictor of poor HIV outcomes as well as forward transmission. Despite this, objective assessment of MA use is suboptimal; self-report can be skewed and short-term markers of MA use (e.g., urine tests) have limited sensitivity. We hypothesize that cumulative assessment of hair MA in people at risk of or living with HIV (PWH) who report at least weekly MA use will increase detection of MA use.

Methods: Leveraging the UCSF Hair Analytical Laboratory’s expertise in the use of hair samples to monitor long-term antiretroviral adherence, we developed a hair analytical method to measure past-month MA use. We then examined stored samples from The mSTUDY, a well-established cohort of MSM that collected self-reported substance use data using validated tool (ASSIST), urine and hair specimens. We analyzed hair from participants who reported daily or weekly MA use but were MA urine test negative at the time of hair collection. Per Society of Hair Testing guidelines, hair was considered MA-positive if concentration was >200 picograms/mg. We defined sensitivity as proportion of positive hair samples from MSM who reported at least weekly use, despite negative urine tests. To determine specificity, we randomly selected n=22 hair samples from controls who reported no MA use and where urine testing was negative urine tests. To determine specificity, we randomly selected n=22 hair samples from controls who reported no MA use and where urine testing was negative urine tests. To determine specificity, we randomly selected n=22 hair samples from controls who reported no MA use and where urine testing was negative urine tests.

Results: Demographics and HIV status of the 44 MSM included in the analysis are presented in the Table. Of the 22 MSM who reported daily or weekly MA use but had negative urine tests, 3 (14%) reported daily urine use and 19 (86%) reported weekly use. Hair analysis detected MA use above the established threshold in 17 additional individuals, yielding a sensitivity of 77% (95% CI 60–95%). Among the 22 control subjects with no reported MA use and negative urine tests for all substances, 3 were found to have MA-positive hair samples, with a specificity of 85% (95% CI 72–100%).

Conclusion: For PWH with a high degree of structural vulnerability, retention in MOUD was low (<30%) despite the availability of low-barrier addiction services in a program serving PWH with homelessness. As all patients retained on MOUD were on methadone, it is crucial to investigate barriers to buprenorphine retention and to enhance methadone clinic referrals from primary care. Finally, addressing the widespread stimulant use observed in this sample of PWH experiencing homelessness is a top priority.

The Opioid Use Disorder Care Cascade for PWH Experiencing Homelessness in Low-BARRIER HIV Care

Ayesha Appa, Gabriela Steiner, Matt Hickey, Elizabeth Imbert, Cayce Cullen, John Friend, Rodrigo Avila, Jai Jackson, Pierre-Cedric Crough, Jon Oskarsson, Francis Mayorga-Munoz, Janet Grochowski, Monica Gandhi
University of California San Francisco, San Francisco, CA, USA

Background: Among people with HIV (PWH) with co-morbid opioid use disorder (OUD), initiation and retention in OUD treatment (e.g., buprenorphine, methadone) substantially reduces overdose deaths and improves HIV viral suppression. We sought to characterize the OUD care cascade for viremic PWH experiencing homelessness enrolled in a low-barrier HIV primary care model with embedded addiction support services.

Methods: The POP-UP clinic at Ward 86 provides comprehensive primary care for viremic PWH with housing instability using a drop-in model with interdisciplinary services including counseling, same-day buprenorphine initiation, methadone clinic in an adjacent building, and on-demand Addiction Medicine consultation. We conducted a retrospective chart review of enrolled patients from February 2019 to July 2023. We identified those with opioid use upon enrollment and tracked their progression through a previously published care cascade for OUD (Figure).

Results: Among 145 PWH experiencing homelessness enrolled in low-barrier primary care, 138 (94%) used substances (78% methamphetamine, 17% opioids, 10% cocaine). Of the 17% who used opioids (25/145), 84% identified as cisgender men, 58% identified as White, 20% Latine, and 16% Black. Almost all opioid use was fentanyl (24/25 fentanyl, 1/25 heroin). All 25 patients reported concomitant use of methamphetamine. Median follow up time was 28 months (interquartile range 12 - 39). Of n=25, 80% (20) were diagnosed with OUD and offered methadone or buprenorphine (i.e., engaged in OUD-specific care); 68% (17) initiated MOUD, but only 28% were retained on MOUD in the subsequent 6 months (Figure). Of n=5 not diagnosed with OUD, n=3 had remote history of OUD, n=2 reported unintentional exposure to fentanyl (with clinical opioid overdose). Of PWH retained on MOUD, 100% were on methadone. HIV viral suppression in PWH retained on MOUD was 86% (6/7) vs. 56% (10/18) HIV viral suppression in PWH with opioid use not initiated or retained on MOUD.

Conclusion: For PWH with a high degree of structural vulnerability, retention in MOUD was low (<30%) despite the availability of low-barrier addiction services in a program serving PWH with homelessness. As all patients retained on MOUD were on methadone, it is crucial to investigate barriers to buprenorphine retention and to enhance methadone clinic referrals from primary care. Finally, addressing the widespread stimulant use observed in this sample of PWH experiencing homelessness is a top priority.
Trends and Correlates of Methamphetamine Use Among Men Who Have Sex With Men in the US, 2014-2021

Michael P. Barry, Benjamin Meana, Sarah N. Glick, David Katz, Travis H. Sanchez, Matthew Golden, Steven M. Goodreau

University of Washington, Seattle, WA, USA, Emory University, Atlanta, GA, USA

Background: Methamphetamine (MA) is a powerful, addictive stimulant drug disproportionately used among men who have sex with men (MSM) in the United States (US). MA facilitates HIV acquisition and undermines HIV care engagement, threatening the US’s goal of Ending the HIV Epidemic. Using a large, national dataset, we examined trends and correlates of MA use among US MSM to identify which subpopulations of MSM in the US have the highest use burden.

Methods: We used data from the 2014-2021 American Men’s Internet Survey, a serial, cross-sectional, web-based survey of cisgender MSM in the US conducted by PRISM Center at Emory University. Each year, approximately 10,000 MSM participate and provide behavioral and health data on the 12 months preceding the survey, including non-injection drug(s) used. We estimated the proportion of MSM who reported non-injection MA use in the past 12 months during each survey year. We used univariate log binomial regression to identify correlates associated with MA use and, with those variables, constructed a multivariate model. As a significantly higher proportion of MSM reported MA use in 2021 than in prior years, we repeated this analytic approach using data from only 2021.

Results: Between 2014 and 2017, the proportion of MSM who reported last-year MA use remained from 3.1%-3.7%. In 2018, that proportion dropped significantly to 2.3%, then increased to 2.9% in 2020 and more than doubled to 6.2% in 2021 (Figure 1). In the multivariate model including all survey years, last-year non-injection MA use had notable associations with: living with HIV and using antiretroviral therapy (ART, PR: 4.83 [4.43, 5.27]); living with HIV and not using ART (PR: 4.38 [3.65, 5.19]); being HIV-negative and using pre-exposure prophylaxis (PR: 1.54 [1.37, 1.72]); and Indigenous race (PR: 1.39 [1.18, 1.62]).

Conclusions: From 2014-2020, non-injection MA use was largely stable, followed by a sharp increase in 2021. MSM living with HIV reported the highest use burden throughout the period. Those who did not use ART reported notably high rates of MA use, signaling potential for HIV transmission to their partners. Our findings that MA use increased after the first year of the COVID-19 pandemic suggests that there is an increased need for MA prevention efforts among MSM. Future work should explore the extent to which MA use has remained high post-pandemic.

Figure 1: Percentages of Men Who Have Sex With Men Reporting Non-injection Methamphetamine Use in the Last Year American Men’s Internet Survey, 2014-2021 (N=1,019)
between hazardous alcohol use and detectable viremia among women recently diagnosed with HIV (WLWH) in Uganda.

**Methods:** We analyzed data from women in the standard-of-care control arm of ThePATH (Providing Access To HIV Care/Ekkudo Study, a cluster-randomized controlled trial of an enhanced linkage to HIV care intervention in rural Uganda. To analyze temporal associations between hazardous alcohol use (Alcohol Use Disorder Test- Concise, AUDIT-C, score ≥ 3) and subsequent detectable viral load (VL) (>20 copies per mL), we built prospective logistic regression models using 6- and 12-month follow-up data. Models were adjusted for age, baseline VL, wealth index and if participants were diagnosed pre/post universal test and treat (Utt). We also ran the model adjusting for self-reported adherence using a question form the Adult AIDS Clinical Trials (AACTG) adherence instrument.

**Results:** Our analytic sample included 128 women who were diagnosed with HIV at study enrollment and had VL data available at follow-up. Median age of participants was 25 years (IQR 21-32); 31% were enrolled before UTT. Prevalence of hazardous alcohol use was 18% at 6-months follow up. The majority (85%) self-reported they were ART adherent at 12- months. In adjusted models, women who report hazardous alcohol use at 6-months had 2.84 greater odds of a detectable VL at 12-months (aOR 2.83, 95% CI 1.05, 7.62, p=0.039). When also controlling for self-reported adherence to ART at follow-up, the magnitude of this relationship is stronger, with women who reported hazardous alcohol use having four times greater odds of a detectable VL at 12-months (aOR 4.07, 95% CI 1.19, 13.8, p=0.025).

**Conclusion:** Our study provides insight regarding the relationship between hazardous alcohol use and detectable viremia among WLWH. While our adherence measure has limitations (e.g., short time frame), our findings suggest alcohol use may be impacting viremia, underscoring the need to screen for and intervene on hazardous alcohol use among WLWH.

**1002 Biomarker Measure Strengthens Alcohol Use Association With HIV Viral Non-Suppression in PWH on ART**

Cristina Espinosa Da Silva1, Robin Fatch1, Winnie Mayindike2, Evgeny Krupitsky3, Nneka Ememunya4, Sarah Puryear4, Aaron Scheffer4, Kako So-Armah4, Gabriel Chamie4, Brian Beesiger4, Kata Marsen4, Frank Paletta4, Phyllis Tien4, Judith Hahn5, for the MACS WIHS

**Background:** Alcohol use among persons with HIV (PWH) predicts poor antiretroviral (ART) adherence and other co-morbidities, but its association with HIV viral non-suppression is inconsistent. This may be due to the limitations of alcohol self-report. The biomarker phosphatidylethanol, PEth, is an objective blood-based alcohol measure correlated with past month alcohol use. We assessed the association between alcohol use (using a composite of PEth and self-report) and HIV viral non-suppression.

**Methods:** We pooled data from PWH on ART for ≥6 months from three RCTs and three observational studies conducted from 2012-2022 in the US, Russia, and Uganda, and measured alcohol use via PEth and the Alcohol Use Disorders Identification Test – Consumption (AUDIT-C) self-reported screener. We categorized PEth/AUDIT-C alcohol use as: (1) no/low risk (PEth<50 ng/mL and AUDIT-C 0-2/2; women 0-3/3); (2) moderate risk (50<PEth<200 ng/mL and AUDIT-C 3-5/women or 4-5/men); (3) high risk (PEth≥200 ng/mL and AUDIT-C≥6); If PEth and AUDIT-C were discordant, the higher-risk group was selected. We used mixed effects logistic regression to model the associations between alcohol use and HIV viral non-suppression (≥250 copies/mL) adjusting for sex, age, ART regimen, other substance use, study design and location. We accounted for within-individual and between-study clustering, examined associations using PEth and AUDIT-C alone, and explored interaction by the above variables.

**Results:** Among 2343 participants (4137 obs), 51% were male, 8% were HIV virally non-suppressed, median PEth was 53 ng/mL (IQR=9-281), and median AUDIT-C was 4 (IQR=1-7). We found a significant association between PEth/ AUDIT-C and HIV viral non-suppression (adjusted OR [95% CI]: high vs. no/low risk = 1.88 [1.07-3.11]). The associations were stronger for PEth/AUDIT-C and PEth alone versus AUDIT-C alone (see Table). We found significant interaction by sex (p<0.10) with a stronger association between high-risk alcohol consumption and HIV viral non-suppression among men compared to the association in women, but no other interaction.

**Conclusion:** These findings suggest that alcohol use measured by a composite of biomarker/self-report is associated with HIV viral non-suppression, particularly among men with HIV on ART. Associations using both PEth/ self-report and PEth alone were stronger than when using self-report alone. Objective measurement of alcohol use may lead to more accurate findings regarding the relationship between alcohol use and HIV outcomes.

**1003 Associations of Alcohol Consumption With Long-Term Mortality of ART-Naive Persons Seeking HIV Care**

Daniel Fuster1, Paola Zuñiga1, Enric Abelli-Deulofeu1, Laura Bermejo1, Santiago Moreno1, Lucio J. García-Fraile1, Ines Suarez-Garcia1, David Vinuesa1, Vicente Estrada1, Julian Olalla-Sierra1, Joan Macias1, Inma Jarrin2, Robert Muga3

1Hospital Germans Trias i Pujol, Barcelona, Spain; 2Hospital Universitario de Ourense, Madrid, Spain; 3Hospital Universitario Reina Sofia, Cordoba, Spain; 4Hospital Universitario de La Princesa, Madrid, Spain; 5Hospital Donostia, San Sebastian, Spain; 6Institute of Health Carlos III, Madrid, Spain; 7Hospital Universitario Reina Sofia, Cordoba, Spain; 8Hospital Universitario de La Princesa, Madrid, Spain; 9Hospital Costa del Sol, Marbella, Spain; 10Hospital Universitario de Valencia, Valencia, Spain; 11Hospital Universitario de Murcia, Murcia, Spain

**Background:** Alcohol consumption >40 grams/day (HR 4.28; 95% CI: 3.13-5.83) was associated with mortality. In the overall sample (1003 participants), crude mortality rate of those who drank >40 grams/day was 3.1% (95% CI: 2.0-4.6%). Among PWID (HR: 4.72; 95% CI: 3.64-6.17) were associated with mortality. In the unadjusted analysis, alcohol consumption >40 grams/day was higher among PWID (26%) than among MSM (24%) or heterosexuals (5%) among individuals enrolled between 2004 and 2021 in 47 HIV/AIDS units. We analyzed crude and adjusted mortality rates by alcohol consumption at first visit. Cox proportional hazard models were used to assess the association between excessive alcohol consumption (>40 gr/day) and mortality after controlling for confounders [i.e., age at first visit, sex, HIV infection (EIA+), CD4 cell count and HIV-RNA viral load and persons who inject drugs as mode of HIV acquisition (PWID)].

**Results:** 8,378 participants (15% women) were included; median age at first visit was 37 years (IQR 29-44 years). Men who had sex with men (MSM) accounted for 61% of the participants, 28% were heterosexuals and 6.3% were PWID. Among drinkers (38% of the study population), mean alcohol consumption was 70 grams per week (± 30.6 gr). In addition, 4.3% of participants reported alcohol consumption >40 grams/day. The prevalence of alcohol consumption >40 grams/day was higher among PWID (26%) than among MSM (24%) or heterosexuals (5%). Prevalence of HIV infection was 8.8%, median log RNA HIV load was 4.54 (IQR: 3.67-5.13) and median CD4 count was 398 cells/μL (IQR: 299-596). After a median follow up of 5.6 years (IQR: 2.5-9.8 years) and a total follow up of 52,799 person-years (p-y), 267 participants (3.1%) had died. Crude mortality rate of those who drank >40 grams/day was 2.09/100 p-y vs. 0.43/100 p-y among those who drank <40 grams/day (HR 4.85 (95% CI: 3.56-6.62), p<0.01). In the unadjusted analysis, alcohol consumption >40 grams/day (HR 4.28; 95% CI: 3.13-5.83), age greater than the median (HR: 2.16; 95% CI: 1.62-2.89), HIV infection (HR: 5.05; 95% CI: 3.94-6.48), CD4 cell count below the median (HR: 2.58; 95% CI: 1.96-3.40) and PWID (HR: 4.72; 95% CI: 3.64-6.17) were associated with mortality. In the adjusted model, alcohol consumption >40 grams/day was associated with a higher risk of mortality (HR 2.39 (95% CI: 1.68-3.42), p<0.01).

**Conclusion:** In this cohort of ART-naive individuals entering HIV care, unhealthy alcohol use was associated with reduced survival.
Pre-Pandemic Patterns of Cannabis Use Among Women With HIV in the United States

Richard J. Wang1, Brooke Bullington1, Phylis Tien1, Bhat Surya1, M. Bradley Drummond2, Gypsypamber D’Souza3, Robert Forony1, Antonina Foster1, Deepa Lazarou1, Anjali Sharma1, Kathleen Weber1, Deborah Jones Weiss3, Danielle F. Haley3, Andrew Edmonds1

1University of California San Francisco, San Francisco, CA, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 3University of Alabama at Birmingham, Birmingham, AL, USA, 4The Johns Hopkins University, Baltimore, MD, USA, 5State University of New York Downstate Medical Center Downstate Medical Center, Brooklyn, NY, USA, 6Emory University, Atlanta, GA, USA, 7Georgetown University, Washington, DC, USA, 8Albert Einstein College of Medicine, Bronx, NY, USA, 9Cook County Health & Hospitals System, Chicago, IL, USA, 10University of Miami, Miami, FL, USA, 11Boston University, Boston, MA, USA

Background: The regulatory environment for marijuana and other cannabis products is changing quickly across the United States, and use has increased over the past decade. The contemporary prevalence of use, frequency of use, and uptake of newer cannabis products, such as vape products, is unknown for women with HIV. Because cannabis products can pose health risks and have immunomodulatory effects mediated by cannabinoid receptors, it is important to understand patterns of cannabis use among women with HIV.

Methods: This analysis includes 1,246 women with HIV enrolled in the Women’s Interagency HIV Study (WHIS) who attended three semi-annual study visits between April 2018 and September 2019. Data on cannabis use were collected at each visit. A Sankey diagram illustrates the flow of participants between different categories of use frequency over the follow-up period.

Results: The period prevalence of cannabis use was 27%, 26% reported smoking marijuana, 8% reported enteric consumption of cannabis products, and 5% reported vaping cannabis products. The prevalence of daily cannabis use was 15%. There was no significant difference in the prevalence of use between sites in states with laws permitting adult or medical use and in states without. Cannabis users were younger, less likely to be employed, and more likely to have used tobacco, alcohol, and other substances. The proportion of users who reported vaping cannabis products increased from 10% in 2018 to 14% in 2019. Compared to cannabis users who did not vape, cannabis users who vaped were more likely to be employed and have a college degree. They were less likely to have smoked cigarettes and more likely to have used e-cigarettes. They were more likely to attend a study site in a permissive jurisdiction. While half of all users reported the same frequency of cannabis use at all three visits, the remaining half of users reported different frequencies of use on at least two visits (Figure 1).

Conclusion: In a cohort of women who are representative of women with HIV in the United States, we found a high prevalence of cannabis use in the period immediately preceding the COVID pandemic. Our prevalence estimate is higher than in the general US population and higher than for women with HIV in the WHIS a decade prior, which was 14%. From 2018 to 2019, there was a small increase in the proportion of users who vaped cannabis products. It was not uncommon to report different frequencies of cannabis use at different study visits across 18 months of follow-up.

Incarceration, Drug Use, and HIV: Optimizing Services for Women Who Use Drugs in Tanzania

Kaitlyn Atkins, Haneefa T. Saleem

The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: Women who use drugs (WWD) in sub-Saharan Africa (SSA) face elevated HIV risk, which is linked to structural vulnerability including high rates of incarceration and arrest. However, little is known about the experiences of formerly incarcerated WWD in SSA or their drug use and HIV outcomes.

Methods: Using respondent-driven sampling (November 2018-February 2019), we recruited WWD reporting past-month heroin use in Dar es Salaam, Tanzania and administered a structured survey (n=200). We described the prevalence of recent incarceration (being in jail or prison in the past six months) and characteristics of recently incarcerated WWD. We used modified Poisson regression with robust variance estimation to calculate prevalence ratios (PRs) and 95% confidence intervals (CI) for the associations between recent incarceration and HIV and drug use outcomes, adjusting for age, education, and duration of heroin use in years.

Results: Over half of WWD (n=119, 61%) reported incarceration in the past six months. The most common reasons for arrest were using drugs (47%) and selling sex (27%). In bivariate analyses, incarceration was associated with transactional sex, symptoms of depression and anxiety, physical violence victimization, and drug use stigma from family and healthcare providers (Table). In adjusted analyses, incarceration was associated with 46% higher prevalence of concurrent sexual partnerships (PR 1.46, 95% CI 1.17-1.82), five times the prevalence of concurrent stimulant use (PR 5.70, 95% CI 1.74-18.70), and 76% higher prevalence of lifetime non-fatal overdose (PR 1.76, 95% CI 1.08-2.85). Among WWD living with HIV, incarceration was associated with missing HIV care appointments (p=0.02).

Conclusion: This study, one of the first to describe HIV-related outcomes among recently incarcerated WWD in SSA, identified converging behavioral and structural risks related to incarceration, which may exacerbate HIV disparities among WWD. Elevated stimulant use among recently incarcerated WWD is of particular concern, given associations with adverse HIV outcomes. In the context of highly criminalized sex work and drug use, interventions that target policing practices such as drug diversion programs, may be effective at reducing incarceration-associated risks. For WWD, including those living with or at risk for HIV, multilevel interventions may be needed to reduce service interruptions and ensure linkage to care during incarceration and reentry.

Differences in Healthcare Access Among Persons Who Inject Drugs by Medicaid Expansion Policy, 2022

Amy R. Baugher, Rashunda Lewis, Lashone Sutter, Maya Haynes, Cyprian Weipert

Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Since 2014, 40 states expanded Medicaid, extending coverage to millions. Persons who inject drugs (PWID) are at increased risk for HIV, often have low income, and could benefit from Medicaid expansion. Yet, many PWID live in non-expansion states, mostly in the South. We compared healthcare access between PWID living in Medicaid expansion vs. non-expansion states. We conducted a subanalysis focused on PWID with HIV.

Methods: We analyzed 2022 data from CDC’s National HIV Behavioral Surveillance in 19 US cities sampling PWID aged 18-64 years (n=6245). We used multilevel log-linked Poisson models to compare PWID’s healthcare access by state-level Medicaid expansion policy (defined as expanding Medicaid by June 1, 2022), producing adjusted prevalence ratios (aPR) and 95% confidence intervals (CI). Significance was determined by CIs overlapping with the null.

Results: Most PWID lived in a Medicaid expansion state (85%). Most PWID in non-expansion states were Black (59%). Only 1 out of 10 PWID in expansion states were uninsured compared to 2 out of 3 PWID in non-expansion states (aPR=0.1, 95%CI=0.1-0.2). PWID in Medicaid expansion states were 5 times as likely to have Medicaid than PWID in non-expansion states (72% vs. 13%; aPR=5.3, 95%CI=5.3-5.3). PWID in Medicaid expansion states were more likely than those in non-expansion states to have visited a doctor in the past 12 months (76% vs. 66%; aPR=1.2, 95%CI=1.1-1.3) and have a usual source of healthcare (52% vs. 38%; aPR=1.4; 95%CI=1.1-1.9). PWID in expansion states were less likely than those in non-expansion states to have an unmet medical need due to cost (18% vs. 41%; aPR=0.4, 95%CI=0.4-0.5). Only 4% of PWID with HIV in expansion states were uninsured compared to 54% of PWID with HIV in non-expansion states (aPR=0.1, 95%CI=0.0-0.1). PWID with HIV in expansion states were less likely than those in non-expansion states to have an unmet medical need due to cost.
states were less likely to have an unmet medical need due to cost than those in non-expansion states (12% vs. 38%; aPR=0.3, 95%CI=[0.2,0.5]).

Conclusion: Medicaid expansion is associated with better access to healthcare and fewer unmet medical needs among PWID, a population that often has diverse medical needs and few resources. Non-expansion hinders efforts to end the HIV epidemic and disproportionately affects Black PWID, contributing to racial/ethnic inequities. Clinics in non-expansion states may link PWID patients to services and patient assistance programs to defray costs. States may consider expanding Medicaid.

1007 Unmet Need for Medication for Opioid Use Disorder Before and After the COVID-19 Pandemic

Jacklynn De Leon, Anna Teplinskaya, Dafna Kanny, Senad Handanagic, Dita Broz, Teresa Finlayson, Cyprian Weinert, for the NHBS Study Group
Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: The COVID-19 pandemic substantially impacted harm reduction services for persons who inject drugs (PWID), including access to medications for opioid use disorder (MOUD) that reduce injection frequency, HIV and HCV transmission, and opioid-related overdose mortality. We sought to assess change in unmet need for MOUD among PWID from pre- to post-pandemic.

Methods: PWID were recruited using respondent-driven sampling to participate in CDC’s National HIV Behavioral Surveillance in 19 U.S. cities in 2018 and 2022. This analysis included PWID who were ≥ 18 years and reported injecting drugs and opioid use in the past 12 months. We obtained prevalence ratios (PRs) and 95% confidence intervals (CIs) using log-linked Poisson models adjusted for city and participant network size and clustered on recruitment chain to assess differences in self-reported unmet need for MOUD between 2018 and 2022.

Results: The analysis included 9,282 PWID interviewed in 2018 and 5,882 PWID interviewed in 2022. Overall, fewer PWID reported unmet need for MOUD in 2022 than in 2018 (28% vs. 25%, PR 0.91, 95% CI: 0.85, 0.98). Additionally, unmet need for MOUD decreased from 2018 to 2022 among PWID who most commonly injected opioids (28% vs. 24%, PR 0.86; 95% CI: 0.77, 0.95), PWID who injected more than once a day (30% vs. 27%, PR 0.89; 95% CI: 0.82, 0.98), and PWID who received syringes from an SSP in the past 12 months (28% vs 25%, PR 0.87; 95% CI: 0.78, 0.97).

Conclusion: Decreases in unmet need for MOUD were observed among those at high need for MOUD, specifically those who injected most frequently and most commonly injected opioids. Unmet need for MOUD also decreased among those accessing SSPs, which are important service programs for PWID. Low-threshold, affordable access to MOUD is key to reducing injection-related risk for HIV and other infectious diseases. Despite COVID-19 pandemic-related disruptions in services for PWID, policy changes to federal guidance for MOUD treatment providers or other innovations in service provision may have contributed to increases in access to MOUD. Additional research is needed to assess what factors or potential policies mitigated the effects of service disruptions due to the pandemic on unmet need for MOUD and the resulting impact on HIV and other infectious diseases.

1008 HIV Prevention Service Use Among PWID in Washington, DC, Pre- and Post-COVID Pandemic Eras

Xinyi Li1, Sydney Bornstein1, Manya Magnus1, Kate Dreznner1, Brittani Saafir-Callaway2, Alan Greenberg1, Hannah Latif1, Irene Kuo1
1George Washington University, Washington, DC, USA; 2District of Columbia Department of Health, Washington, DC, USA

Background: The COVID-19 pandemic disrupted access to critical healthcare and HIV prevention services for people who inject drugs (PWID). We explored the pandemic’s potential impact on use of healthcare and HIV prevention services among PWID in the pre- and post-COVID pandemic eras.

Methods: We used data from the 2018 and 2022 National HIV Behavioral Surveillance system in Washington, DC. PWID were recruited via respondent-driven sampling (RDS) and were ≥18 years old, resided in the Washington metropolitan statistical area, and reported injecting non-prescribed drugs in the past 12 months. Self-reported healthcare and HIV prevention service utilization, drug-use behaviors, and drug treatment were assessed. RDS-weighted percentages were calculated; Rao-Scott chi-square tests identified significant differences in service utilization and drug use behaviors comparing 2018 and 2022.

Results: N=511 participants in 2018 and N=229 participants in 2022 were included in the analysis. More than 70% of both samples were male. Compared to 2018, a higher proportion of PWID were >65 years old and fewer identified as Non-Hispanic Black in 2022. In 2022, a higher proportion of PWID reported using ≥2 most frequently injected drugs (6.3% vs. 16.0%, p<0.0001). In 2022, more PWID owned naloxone (35.0% vs. 87.8%, p<0.0001) and sought out fentanyl (10.6% vs. 26.2%, p<0.001) in the past year. No differences were found in having a usual healthcare source, HIV testing, or PrEP awareness and uptake. However, a significantly lower proportion of 2022 participants reported sharing needles (40.5% vs. 21.6%, p<0.01), cookers/cotton/water (53.4% vs. 35.8%, p=0.0058), or syringes (40.8% vs. 23.0%, p<0.01) than those in 2018. Fewer PWID obtained needles from syringe service programs (SSPs) over time (74.8% vs. 59.9%, p=0.03), but a larger proportion in 2022 obtained needles from places other than SSPs or HIV prevention programs (9.4% vs. 23.3%, p=0.01). In 2022, past year engagement in drug treatment programs also increased from 31.4% to 61.6% (p<0.0001).

Conclusion: Significant decreases in injection-related risk behaviors and increases in utilization of drug-related services were observed among PWID in Washington, DC. Changes in drug use behaviors over time and where PWID received services were also observed, suggesting the need to better understand how changes in service utilization and drug use patterns impact PWID health.

1009 Changes in HIV PreP Awareness and Use Among PWID in 19 US Cities, 2018 and 2022

Johanna Chapin-Bardales, Dita Broz, Patrick Eustaquio, Jonathan Feelemyer, Teresa Finlayson, Savannah Harris, Cyprian Weinert, for the NHBS Study Group
Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: HIV pre-exposure prophylaxis (PrEP) awareness and use among persons who inject drugs (PWID) has been low since the introduction of PrEP in the U.S. in 2012. Limited data are available to monitor PrEP awareness and use specifically among PWID.

Methods: In 19 U.S. cities participating in 2018 and 2022 National HIV Behavioral Surveillance, PWID were recruited using respondent-driven sampling and offered a behavioral survey and HIV testing. We examined the prevalence of PrEP awareness and PrEP use in the past 12 months, overall and by key characteristics, among HIV-negative PWID in 2018 and 2022. We obtained adjusted prevalence ratios, 95% confidence intervals, and p-values using log-linked Poisson models accounting for clustering by recruitment chain and adjusting for city and participant network size to assess changes in PrEP outcomes over time.

Results: From 2018 to 2022, PrEP awareness among PWID increased from 25.6% to 35.3% (p<0.01), yet PrEP use in the past year remained stable at 1.2% (p=0.35). The approximate 10 percentage-point increase in PrEP awareness between 2018 and 2022 was consistent across demographic and behavioral subgroups. PrEP awareness increased significantly among PWID who had receptively shared syringes, shared injection equipment, had condomless vaginal or anal sex, and had a bacterial STI in the past year (all p-values <0.01). Minimal yet significant increases in PrEP use were observed for those who had receptively shared syringes, shared injection equipment, and received a recent HIV test (all p-values <0.05); nevertheless PrEP uptake in these groups did not surpass 2%. PrEP use was highest among those reporting past-year male-male sex and bacterial STI (both 4.8% in 2022); this was stable compared with 2018.

Conclusion: PrEP awareness significantly increased from 2018 to 2022 among PWID. Still, only about one-third were aware of PrEP in 2022. Increases in awareness were consistent across subgroups; suggesting that PrEP messaging is reaching groups with a greater risk of HIV acquisition, yet changes may be due to a generalized increase in awareness overall. PrEP use remained suboptimal. PWID at higher risk of injection-related HIV acquisition and those who obtained HIV-related services did experience significant increases in PrEP uptake unlike other subgroups; however, increases were small. Efforts to improve PrEP messaging, provider training, and access specifically for PWID may serve to further increases in PrEP awareness and use.

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1010 Changes in PrEP Awareness Among Men Who Have Sex With Men and PWID: 19 US Cities, 2018 and 2022

Patrick Eustaquio, Janet Burnett, Ranthane Marcus, Joseph Prejean, Johanna Chapin-Bardales, Susan Cha, for the National HIV Behavioral Surveillance Study Group

Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: HIV preexposure prophylaxis (PrEP) is effective at preventing infection among persons at risk for acquiring HIV. PrEP campaigns have focused on sexual behaviors, particularly same-sex behaviors, and may not be reaching heterosexual-identifying men or people who inject drugs. This analysis aimed to explore changes in PrEP awareness and use among men who have sex with men and inject drugs (MSM-ID) in 2018 and 2022 by sexual orientation.

Methods: We analyzed data from 2018 and 2022 National HIV Behavioral Surveillance among people who inject drugs recruited via respondent-driven sampling in 19 US urban areas. The analytic sample was restricted to HIV-negative males who inject drugs and who had sex with another man in the last 12 months (MSM-ID). Using log-linked Poisson regression models, adjusted prevalence ratios (aPRs) and 95% confidence intervals (95% CIs) were calculated to estimate changes in PrEP awareness stratified by sexual orientation. Models were adjusted for age, race/ethnicity, city, and network size, and accounted for clustering by recruitment chain. Small sample size of PrEP users precluded statistical comparison on PrEP use.

Results: Of HIV-negative MSM-ID, 71.5% (331/463) in 2018 and 66.8% (197/295) in 2022 identified as gay/bisexual. Between 2018 and 2022, there was a significant increase in PrEP awareness among gay/bisexual MSM-ID (45.5% in 2018, 64.5% in 2022; aPR=1.51, 95% CI=1.32-1.72) but not for heterosexual MSM-ID (39.4% in 2018, 40.8% in 2022; aPR=0.98, 95% CI=0.73-1.31) (Figure). Among MSM-ID aware of PrEP, PrEP use was low in both years regardless of sexual orientation (gay/bisexual: 15.3% in 2018, 10.2% in 2022; heterosexual: 7.7% in 2018, 2.5% in 2022).

Conclusion: The increase in PrEP awareness was limited to gay/bisexual men, however, PrEP use was low overall among MSM-ID regardless of sexual orientation. Without adequate HIV prevention, MSM-ID may be at risk of acquiring or transmitting HIV through injecting drugs and sex. Provider training may be useful to encourage assessment of both injection- and sex-related indications for PrEP during provider visits. Educational campaigns tailored to MSM-ID are warranted, particularly around risk behavior and benefits of PrEP regardless of one’s sexual orientation.

1011 RDS Network Size Among People Who Inject Drugs in Kenya as a Predictor of HIV and HCV Positivity

Matthew Akiyama1, Amihossom Alvandi1, Krista Gile1, Yun Jiang1, Lindsey Riback2, Mercy Nyakwola3, Jibeet Boit3, Rose Wafuila4, Nazila Ganatra5

1Albert Einstein College of Medicine, Bronx, NY, USA; 2University of Massachusetts, Worcester, MA, USA; 3Ministry of Health, Nairobi, Kenya

Background: People who inject drugs (PWID) are a key population for HIV and HCV transmission in sub-Saharan Africa. Understanding transmission networks is critical to reducing prevalence and incidence among PWID. Yet, network-related factors associated with seropositivity are poorly understood.

Methods: We are recruiting PWID using respondent driven sampling (RDS) from syringe services programs in Kenya. Upon enrollment, participants complete biobehavioral surveys, receive HIV, HCV, and HBV testing, and are provided with 3 coupons to recruit their peers who are not already in the study. Upon return of 3 coupons, 5 tokens are given to establish cross ties among PWID who are already enrolled in the study. We evaluated the relationship between RDS-network size and HIV and HCV infection using t-tests and permutation tests.

Results: Among 2135 PWID enrolled thus far from May 2022 to August 2023, participants are mainly male (89.8%) and 34.6 years old (SD=±8.7) on average. 236/2135 (11.1%) are HIV positive; with highest regional prevalence in the Coast (130/962, 13.5%), followed by Nairobi (83/770, 10.8%), and Western Region (21/393, 5.3%). HCV follows a similar gradation moving inland — 425/2135 (19.9%) overall; 248/962 (25.8%), 73/770 (22.7%) and 2/393 (0.5%), respectively. On average, participants report they know 11.1 (SD=±14.9) PWID who also know them among whom they have seen 8.4 (SD=±6.2) in the last 30 days and have injected with 5.4 (SD=±5.4). The average network size as determined by coupon and token recruits was 2.2 with 7 (0.3%) having >10 connections. We observed a highly significant positive relationship between the number of RDS connections and likelihood of HIV/HCV co-infected vs uninfected (p<0.001), HIV-monoinfected vs uninfected (p<0.001), and HCV mono-infected vs. uninfected (p<0.05).

Conclusion: These data, while preliminary, may inform policy makers and programs on HIV and HCV treatment and prevention strategies among PWID. The robust link between network connectivity and elevated HIV and HCV risk highlights the importance of network dynamics in disease transmission. Further study is needed on the potential efficiency of network-based interventions compared with traditional testing and linkage to care to advance efforts toward ending the HIV epidemic and HCV elimination.

1012 HIV Incidence & Changes in Network Structure Among PWID in New Delhi Following the COVID-19 Pandemic

Steven J. Clipman1, Shrutl Mehta1, Aylur K Srikrishnan1, Katie Zook2, Shobha Mahapatra3, Maniratnam S. Kuma1, Gregory M. Lucas1, Carl Latkin3, Sunil Suhas Solomon3

1The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 3YR Carlos Verde qualidade for AIDS Research and Education, Chennai, India

Background: Identifying transmission predictors among people who inject drugs (PWID) is vital for curbing HIV spread. We previously described the role of a particular venue on transmission of HIV among PWID in New Delhi. COVID-19 lockdowns forced PWID at this venue to return home or relocate. The impact of such regulations on HIV transmission remains unknown.

Methods: From 2017-19 (Pre-COVID), 2,512 PWID were recruited into a dynamic cohort and followed semi-annually until March 2020. Over 1,000 participants were lost (migrated or deceased) when the site re-opened. An additional 987 PWID were recruited to replace lost participants using identical procedures between February 2022 and April 2023 (Post-COVID). In both samples, indexes initiated sampling; they recalled who they injected with in the past month and recruited them. Similarly, each recruit named and recruited their recent injection network. Biometric data was used to identify duplicates and establish cross-network links. Injection venues were captured via a survey. Individual and network factors were analyzed for associations with HIV seroconversion using Poisson regression.

Results: Since 2017, 3,499 PWID were recruited; 37.2% were living with HIV (37.0% and 37.6% in the pre- and post-COVID recruits, respectively). Among those without HIV, median age was 28 years; 98% were male, 69% reported daily injection and 48% reported needle sharing in the prior 6 months. 243 seroconversions were observed over 1912.5 person years (HIV incidence of 12.7 per 100 p-y); incidence in the pre- and post-COVID recruits was 13.8 and 7.3 per 100 p-y, respectively. In pre-COVID recruits, individual and network factors, and one particular venue were predictors of seroconversion. In post-COVID recruits, depression, needle sharing, network distance to an HIV-positive person, and 19 venues were associated. Further, venues associated with seroconversion in the
new recruits differed from those pre-COVID (Figure). The post-COVID network was sparser with greater diameter, path length, and fewer injection partners, featuring linear recruitment chains and fewer cross-network links.

**Conclusion:** Post-COVID, the network structure of PWID was altered. HIV seroconversion in the post-COVID recruits was more strongly driven by spatial ties rather than individual-level behavior, though behaviors were largely comparable to the original cohort. The diffusion of incidence across several newer venues could signal an impending outbreak of HIV at venues that were previously low risk.

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**1013 Spatial Clustering of HIV Viremia Among People Who Inject Drugs (PWID) in India**


1 The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2 The Johns Hopkins University, Baltimore, MD, USA, 3 JFK Graduate Center for AIDS Research and Education, Chennai, India, 4 The Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Background:** While India has made significant progress in engaging people on antiretroviral therapy (ART), people who inject drugs (PWID) living with HIV (PLWH) remain under-engaged in treatment with only ~26% on ART and 32% virally suppressed. Using geospatial data to identify geographical clusters of viremia could help find optimal locations to intervene.

**Methods:** Respondent-driven sampling was used to enroll a sample of ~750 PWID in New Delhi from April-August 2023. Participants were ≥18 years and reported injection drug use in prior 2 years. Participants underwent HIV testing and provided up to 5 locations where they injected in the prior year. Viral load testing was conducted on all positive specimens. We mapped locations where PWID injected and used scan statistics to assess spatial clustering of HIV viremia (>1000 copies/mL). We explored distance from treatment centers (ARTCs) among PLWH and sociodemographic/risk behaviors associated with reporting injecting at a cluster location among PLWH with viremia using Fisher’s exact test and logistic regression.

**Results:** Among the 752 enrolled PWID, median age was 27, 99% were male, 66% experienced recent homelessness, 74% inject daily, and 32% of PLWH were viremic. Participants injected in places a median distance of 2.4 km (IQR, 2.3 – 2.6) from ARTCs. This did not vary by viremia status. Only 39% of PLWH were previously diagnosed. Seven clusters were identified, and one (C1) was statistically significant, located near ‘Old Delhi’ (Figure 1). A higher proportion of PWID with viremia injecting in C1 reported injecting daily (94% v. 72%), missing treatment due to drug use (75% v. 29%), and a larger network size (median 60 v. 12 PWID) compared to those not in C1. PWID with viremia who injected in C1 also had higher proportions of moderate/severe depression (50% v. 21%) and of injecting with multiple persons over half the time (72% v. 37%).

**Conclusion:** In an urban setting with a growing HIV epidemic among PWID, there was a geospatial cluster of PWID with untreated HIV associated with higher levels of risk behaviors — potentially facilitating transmission. Most PWID injected daily and at least 2km from ARTCs. Finding additional areas of high viremia using other point pattern and spatial autoregressive techniques can further assess spatial associations with ARTCs. Identifying distinct geographic areas characterized by high viremia can provide targets for field-based mobile strategies incorporating testing, prevention and treatment to interrupt transmission.

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**1014 HIV-1 Transmission Dynamics Among People Who Inject Drugs on the US/Mexico Border Amid COVID-19**

Britt Skaathun, Steffanie Strathdee, Cho-Hee Shrader, Carrie Nacht, Annick Borquez, Inna Artamounova, Alicia Harvey-Vera, Carlos Vera, Gudelia Rangel, Caroline Ignacio, Brendon Woodworth, Antoine Chaillon, Tetyana Vasylyeva

1 University of California San Diego, La Jolla, CA, USA, 2 Columbia University, New York, NY, USA, 3 University of California San Diego, San Diego, CA, USA, 4 United States-México Border Health Commission, Tijuana, Mexico

**Background:** We assessed HIV prevalence, incidence, and patterns of phylogenetic clustering and cluster growth in networks among people who inject drugs (PWID) in the U.S./Mexico border region during the COVID-19 pandemic.

**Methods:** We conducted secondary analysis of a longitudinal study of PWID aged ≥18 years from 3 groups: PWID who live in San Diego county (SD) and engaged in cross-border drug use in Tijuana ≤24 months ago (SD CBDUs), and PWID who did not engage in cross-border drug use (NCBDUs), and who live in either SD or Tijuana (TJ). Between Oct/2020-Oct/2021, participants underwent semi-annual surveys and provided samples for HIV. We calculated HIV prevalence and bivariate incidence-density rates (IR) between baseline and 18-month follow-up visits. Bayesian phylogenetic analysis was used to identify local transmission clusters and estimate their age (as time to most recent common ancestor, TMRA). We further applied a birth-death skyline model to estimate changes in the effective reproductive number of large local transmission clusters (Re).

**Results:** At baseline (n=612), HIV prevalence was 8% (4% SD CBDU, 4% SD NCBDU, 16% TJ NCBDU). Of HIV-seronegative PWID at 18 months follow-up, 9 HIV seroconversions occurred (IR: 1.357 per 100 person-years (95% CI: 0.470, 2.243). 7 of whom were TJ NCBDU and 2 were SD CBDU. We identified 16 phylogenetic clusters that included at least 1 sequence from the cohort (cluster size range 2-17), among which 9 (56%) had sequences from both the U.S. and Mexico. Three studies had highest posterior density (HPD) intervals of TMRA estimated to overlap with the COVID-related US-Mexico border closure in 2020 and all included participants from both sides of the border. One of the two identified large clusters (N=15) included TJ NCBDU and SD CBDU participants only, 47% of the cluster reported sex work, and continued to grow during the border closure (Re=4.8, 95% HPD 1.5 – 9.1).

**Conclusions:** During the COVID-19 pandemic, HIV phylogenetic clusters were detected with sequences from both sides of the border, one of which continued to grow despite the border closure. Despite relatively low HIV incidence overall, mobile harm reduction services providing syringes and HIV testing, and coordination with municipal HIV programs to initiate ART and PrEP are needed to reduce transmission, particularly in Tijuana.
1015 HIV-1 Outbreak Among PWID in Thessaloniki: Recent Expansion in the Midst of COVID-19 Pandemic

Evangelia G. Kostaki, F. Chatzopoulou 1, S. Roussou, E. Tsirgianni, M. Psichogiou 1, L. Goulis 2, L. Skoura, S. Metallidis 1, C. Tsiaras 1, G. Magierkinis 1, A. Beloukhas 1, A. Hatzakis 1, V. Spyra 1, D. Chatzidimitriou 1, D. Paraskevis 1

1University of Athens, Athens, Greece, 2 Aristotle University of Thessaloniki, Thessaloniki, Greece

Background: During the implementation of "ALEXANDROS", a community-based programme, an HIV-1 outbreak was identified among people who inject drugs (PWID) in Thessaloniki, the second largest city in Greece, between 2019 and 2021. Preliminary phylogenetic analysis suggested that 25 sequences from PWID sampled in Thessaloniki during 2019-2021, belonged to 3 phylogenetic clusters of subsubtype A6 and 1 cluster of subsubtype A1. We aimed to investigate the characteristics of this new outbreak by means of molecular epidemiology.

Methods: Phylogenetic analysis was performed for subsubtype A6 including 3 phylogenetic clusters consisting of 3 (2 PWID and 1 non-PWID sequences), 12 (10 PWID and 2 non-PWID sequences), and 19 (11 PWID and 8 non-PWID sequences) sequences from Thessaloniki. Additionally, we included in our analysis 5 PWID sequences obtained between 2010 and 2015 as well as several non-PWID sequences since these 3 phylogenetic clusters were part of a larger monophyletic cluster from Thessaloniki. Phylodynamic analysis was performed by using the Bayesian approach and birth-death skyline serial model in BEAST v2.7.4 program.

Results: Molecular clock analysis showed that the most recent common ancestor (tMRCA) of the largest (N=11) and the second largest (N=10) cluster from PWID, was estimated in the middle of October 2018 and in May 2014, respectively. For the third PWID cluster (N=2), the tMRCA was estimated in July 2017. Phylodynamic analysis showed that the effective reproductive number (Re) started to increase at the beginning of 2019 and remained high (Re > 1) until the end of the study period in the end of 2021. Moreover, the spatial origin of all the clusters was from Thessaloniki.

Conclusion: Our study provides a detailed figure about an HIV-1 outbreak in Thessaloniki. Phylodynamic analysis showed that the time of epidemic growth coincided with the time of the initial identification in 2019. Our findings showed that HIV-1 transmission continues at high rates (Re > 1) among PWID until the end of 2021. Notably, the covid-19 pandemic did not avert HIV-1 transmission among this group. The early origin of the three clusters suggests that HIV-1 virus spreading among this group was previously circulated in the same area. The continuous spread of HIV-1 among this highly vulnerable group is alarming and requires implementation of public health measures.

1016 Disparities in HIV Care Among Hispanic/Latino Persons by Birthplace and SVI: United States, 2021

Juliet A. Morales, Janetta Gant Sumner, Xiaohong Hu, Shacara Johnson Lyons, Anna Satcher Johnson

Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Hispanic/Latino persons (Latino hereafter) comprise 19% of the U.S. population but accounted for 29% of new HIV infections in 2021. Latino persons have historically been treated as a homogenous group in public health research despite their social and cultural diversity. This study aimed to assess birthplace and Social Vulnerability Index (SVI) differences among Latino persons not linked to their HIV care and without viral suppression in the U.S.

Methods: Data from CDC’s National HIV Surveillance System were used for Latino persons aged ≥18 years with HIV diagnosed during 2021. Data were limited to 48 U.S. jurisdictions with complete reporting of laboratory results to CDC. Cases were linked via census tracts to CDC/ATSDR’s SVI. Non-linkage to care was defined as no viral suppression aPR 0.75, 95% CI 0.61–1.00) had a decreased prevalence of non-linkage to care. Persons born in Mexico (non-viral suppression aPR 0.75, 95% CI 0.64–0.87), South America (aPR 0.69, 95% CI 0.57–0.84), and other Caribbean areas (excluding Puerto Rico and Cuba; aPR 0.48, 95% CI 0.30–0.77) had a decreased prevalence of non-viral suppression, compared with those born in the U.S. No significant differences were seen among SVI quartiles, compared to the lowest SVI (lowest vulnerability) quartile, for either care outcome.

Conclusion: Disparities in HIV care outcomes exist within the Latino population by birthplace, with those born in certain non-U.S. areas more likely to be linked to care and have viral suppression soon after diagnosis. Effective interventions that increase care and prevention access must be tailored and expanded for this diverse group.

1017 Prevalence of HIV Testing and HIV Positivity in the Hispanic Community Health Study/Study of Latinos

Mario J. Trejo 1, Jonathan Ross 2, Robert Kaplan 1, Tonia C. Poteat 1, Linda C. Gallo 1, Krista M. Pereira 1, Bonnie E. Shook-Sa 3, Gregory A. Talavera 1, David B. Hanna 1

1Fred Hutchinson Cancer Center, Seattle, WA, USA, 2Montefiore Medical Center, Bronx, NY, USA, 3Albert Einstein College of Medicine, Bronx, NY, USA, 4Duke University, Durham, NC, USA, 5San Diego State University, San Diego, CA, USA, 6University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Although HIV disproportionately affects U.S. Hispanics/Latinos, over 50% of this population has never been tested for HIV. While factors such as healthcare access, English proficiency, acculturation, and nativity likely impact HIV testing among Hispanics/Latinos, few population-based studies have examined these associations. We aimed to describe patterns of HIV testing and test positivity among participants in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL), a population-based longitudinal cohort study of U.S. Hispanics/Latinos which recruited participants between 2006-2011 aged 18-74 years.

Methods: We analyzed data from HCHS/SOL participants who attended study visit 3 (between 2021 and 2023) at field centers in Bronx, NY; Chicago, Miami; and San Diego. Outcomes were lifetime HIV testing, past year testing, and test positivity, by self-report. We estimated prevalence of outcomes by assigned female at birth (AFAB) vs. assigned male at birth (AMAB), sexual orientation, nativity, preferred language, and level of acculturation (measured using the Short Acculturation Scale for Hispanics [SASH] social scale), and calculated 95% confidence intervals.

Results: Among 7074 participants, mean age was 60 years (SD 12.1), 63% were AFAB, 73% spoke Spanish as their primary language, 75% were born outside of the U.S. and had been living in the U.S. for an average of 30 years (SD 11.9), and most reported sexual orientation as straight/heterosexual (91%). In total, 3317 (47%) had ever tested for HIV, 205 (3%) tested within the past year, and 69 (1%) reported testing positive. Ever HIV testing was higher among AFAB vs. AMAB (49% vs 43%; p<0.001), persons identifying as heterosexual vs. homosexual (84% vs 46%; p<0.001), U.S./U.S. territories-born non-U.S. born (56% vs 44%; p<0.001), primary English vs Spanish speakers (61% vs 44%; p<0.001), and highest vs lowest tertiles of social acculturation score (52% vs 42%; p<0.001). Similar patterns were observed with respect to past year HIV testing and test positivity (Table).

Conclusion: In a large, representative study of Hispanic/Latinos at 4 U.S. sites, we observed large disparities in HIV testing and test positivity by nativity, language preference, and level of acculturation. Achieving CDC recommendations for universal lifetime HIV testing among Hispanic/Latinos will require targeted interventions to increase uptake among individuals who are less acculturated.

Table: HIV testing and positivity by nativity, preferred language, and acculturation level in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL)

<table>
<thead>
<tr>
<th>Factor</th>
<th>ever Tested</th>
<th>ever Tested positive</th>
<th>ever Tested positive by nativity</th>
<th>ever Tested positive by preferred language</th>
<th>ever Tested positive by acculturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ever Tested</td>
<td>3317 (47%)</td>
<td>205 (3%)</td>
<td>69 (1%)</td>
<td>0.52 (0.43–0.61)</td>
<td>0.52 (0.43–0.61)</td>
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<tr>
<td>ever Tested by nativity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.S.</td>
<td>3317 (47%)</td>
<td>205 (3%)</td>
<td>69 (1%)</td>
<td>0.52 (0.43–0.61)</td>
<td>0.52 (0.43–0.61)</td>
</tr>
<tr>
<td>U.S. territories-born</td>
<td>3317 (47%)</td>
<td>205 (3%)</td>
<td>69 (1%)</td>
<td>0.52 (0.43–0.61)</td>
<td>0.52 (0.43–0.61)</td>
</tr>
<tr>
<td>non-U.S. born</td>
<td>3317 (47%)</td>
<td>205 (3%)</td>
<td>69 (1%)</td>
<td>0.52 (0.43–0.61)</td>
<td>0.52 (0.43–0.61)</td>
</tr>
<tr>
<td>ever Tested by preferred language</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>English</td>
<td>3317 (47%)</td>
<td>205 (3%)</td>
<td>69 (1%)</td>
<td>0.52 (0.43–0.61)</td>
<td>0.52 (0.43–0.61)</td>
</tr>
<tr>
<td>other</td>
<td>3317 (47%)</td>
<td>205 (3%)</td>
<td>69 (1%)</td>
<td>0.52 (0.43–0.61)</td>
<td>0.52 (0.43–0.61)</td>
</tr>
<tr>
<td>ever Tested by acculturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>highest</td>
<td>3317 (47%)</td>
<td>205 (3%)</td>
<td>69 (1%)</td>
<td>0.52 (0.43–0.61)</td>
<td>0.52 (0.43–0.61)</td>
</tr>
<tr>
<td>lowest</td>
<td>3317 (47%)</td>
<td>205 (3%)</td>
<td>69 (1%)</td>
<td>0.52 (0.43–0.61)</td>
<td>0.52 (0.43–0.61)</td>
</tr>
</tbody>
</table>
1018 Using the ICE Method to Examine Income/Racial Segregation and HIV outcomes: United States, 2020-2022
Wei Song, Mesfin S. Mulatu, Nicole Crepaz, Guoshen Wang, Aba Essuon, Mingjing Xia
Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** The estimated number of new HIV infections in the United States dropped 12% in 2021 compared to 2017, but the racial and ethnic disparities in HIV infection persist. Community-level factors contributing to social vulnerabilities (e.g., poverty, lack of access to healthcare and services) may affect HIV outcomes. This analysis assessed the association between county-level social vulnerability and CDC-funded HIV testing and HIV positivity rates, and whether the association varies by demographic characteristics, testing site type, and Phase I Ending the HIV Epidemic (EHE) jurisdiction status in 2020-2022.

**Methods:** We used testing data submitted to CDC by 60 state and local health departments (HDs) and 117 community-based organizations (CBOs). We combined HIV testing data with the latest county-level composite measure of economic, medical, and social vulnerability captured by the Minority Health Social Vulnerability Index (MHSVI). HIV testing and HIV positivity rates were analyzed by age, gender, race/ethnicity, testing site type, and Phase I EHE jurisdiction status and stratified by low, moderate, and high MHSVI scores. We calculated prevalence ratio (PR) with a 95% confidence interval (CI) to measure relative disparity by comparing county tracts with high vs. low MHSVI scores.

**Results:** In 2020-2022, CDC-funded HDs and CBOs conducted 4,906,507 HIV tests: 113,721 (2.3%) in low, 583,307 (11.9%) in moderate, and 4,209,479 (85.8%) in high social vulnerability counties. Overall HIV positivity rate was 1.03%, including 0.52% in low, 0.63% in moderate, and 0.10% in high social vulnerability counties. HIV positivity rate was higher in than in low social vulnerability counties (PR=2.12; 95% CI=1.95-2.30). In addition, the relative disparity in HIV testing and positivity rates was consistently higher in high MHSVI counties than in low MHSVI counties regardless of age groups, gender, race/ethnicity, testing site type, and the Phase I EHE jurisdiction status.

**Conclusion:** HIV testing supported by CDC funding, and HIV positivity rates are higher in communities characterized by high levels of social vulnerability. These findings suggest that CDC’s HIV testing efforts are directed to the most vulnerable communities and are identifying persons with HIV infection. Continued monitoring of the association between county-level social vulnerability and HIV positivity rates would be informative in guiding HIV testing efforts and resource allocation for achieving EHE goals.

1019 Using the ICE Method to Examine Income/Racial Segregation and HIV outcomes: US, 2021
Zanetta Gant Sumner, André Dailey, Xiaohong Hu, Shacara Johnson Lyons, Anna Satterch Johnson
Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** Assessing the role of segregation on poor health outcomes among Black persons in the United States (U.S.) can inform interventions aimed at increasing health equity. We examined associations between HIV outcomes (diagnoses, linkage to HIV medical care, and viral suppression) and Index of Concentration at the Extremes (ICE) measures for economic and racial segregation in the U.S. in 2021.

**Methods:** Census tract-level data on diagnoses, linkage to HIV medical care, and viral suppression from the CDC’s National HIV Surveillance System were used. Three ICE measures of spatial polarization were obtained from the U.S. Census Bureau’s American Community Survey: ICEincome (Income segregation), ICErace (Black-White racial segregation), and ICEincome+race (Black-White racialized economic segregation). Rate ratios (RRs) for diagnoses, and prevalence ratios (PRs) for linkage to care within 1 month of diagnosis and viral suppression within 6 months of diagnosis were estimated with 95% confidence intervals (CIs). Differences across ICE quintiles were examined using the most privileged communities (Quintile 5, Q5) as the reference group.

**Results:** Across all outcomes and ICE measures, a general pattern of increasingly worse outcomes from most to least privileged quintile was observed. Among all 3 ICE measures, a higher likelihood of HIV diagnosis in Q1 compared with Q5 was observed for ICErace (PR =10.13; CI=9.58–10.70). For HIV diagnosis rates, RRs were consistently greater for ICErace across quintiles. Among all 3 ICE measures for linkage to care and viral suppression, a lower likelihood in Q1 compared with Q5 were observed for ICEincome (linkage, PR =0.94; CI=0.92–0.95; viral suppression, PR =0.89; CI=0.87–0.91), followed by ICEincome+race (linkage, PR=0.94; CI=0.92–0.96; viral suppression, PR=0.90; CI=0.87–0.92). For linkage to care and viral suppression, PRs were consistently lower for ICEincome+race.

**Conclusion:** We found that poor HIV outcomes and disparities were associated with income, racial, and racialized economic segregation as measured by ICE. Persons living in highly segregated and deprived communities experience a lack of access to quality, affordable health care. Expanded efforts are needed to address the social/economic barriers that impede access to HIV care among Black persons. Increased partnerships between government agencies and the private sector are needed to change policies that contribute to and sustain racial and income segregation.

1020 The TIME Study: Trans People Living With HIV Throughout Europe: Clinical Outcomes and Stigma
Jo Smith, José Eduardo de Carvalho Peres, Sujin Kang, Tara Suchak, Lorena de la Mora Cañizo, Amanda Clarke, Tanya Adams, Sally Jewellbury, Yu Tang, Andrea Calcagno, Emanuele Drapper, Anna Serra, Maria Mazzetti, Anna Maria Cattelan, Marta Boffito
for the TIME Study Group
1Clinical and Westminster NHS Foundation Trust, London, United Kingdom; 2Hospital Clinic of Barcelona, Barcelona, Spain; 3University Hospitals Sussex, Brighton and Hove, United Kingdom; 4Manchester University, Manchester, United Kingdom; 5University of Turin, Turin, Italy; 6University of Padua, Padua, Italy

**Background:** Transgender and gender-diverse (TGD) people are at risk for 49x higher risk of acquiring HIV than general populations. We present findings from the TIME study (Transgender people living with HIV throughout Europe), assessing viral response to antiretroviral therapy (ART) in TGD people living with HIV (PLWH) in Europe; exploring demographics, risk behaviours, community needs, barriers and facilitators to ART adherence.

**Methods:** 6 HIV centres in London, Manchester, Brighton, Barcelona, Turin, Padua targeted recruitment of all patients >18 diagnosed with HIV, having ever been prescribed ART, and identifying as TGD. Clinics submitted observational data from medical records on gender identity, HIV viral loads (VL), CD4 counts, ART prescription adherence and comorbidities at enrolment and 6-monthly for 18 months. Participants completed questionnaires on their lifestyles, transitions, self and societal perceptions of gender, HIV, ART, stigma and discrimination.

**Results:** 100 participants were recruited to date, 88% were trans women, of mean age 41 (22-65). 66% had completed medical transition. 60% had one or more physical/mental comorbidities at recruitment; at 18 months 36% did. 25% experienced physical violence in the preceding year. Mean baseline CD4 counts were 685 (100-1846) and 696 (364-1229) at 18 months. 18% of patients had known detectable VLs (<50 copies/ml) at baseline, falling to 11% at 18 months. 5% had clinician-documented ART adherence issues during the study. 30% agreed or strongly agreed that having to take ART worried them. 57% were worried or very worried about long-term effects of ART. 22% found ART disruptive to their lives, and 22% experienced unpleasant ART side effects. 31% felt shame and 27% guilt relating to HIV. 10% described not always having sex as safe as they want, and 27% reported concern about rejection by a partner. 12% had recently received care for suicidal ideation, dropping to 7% at 18 months. 48% described their sex life as better since transition, and 61% the same or better since HIV diagnosis.

**Conclusion:** Although immune status was fair, adherence issues and participant concerns about ART adverse effects were high; VL detectability rates were over 8x higher than in UK PLWH generally. However, a significant proportion of TGD PLWH reported sexual satisfaction since their gender transition and/or HIV diagnosis, indicating that supportive gender and HIV care can promote better quality of life, thus the need for intersectional stigma-aware health service design.

1021 Hazardous Impact of Social Determinants of Health on HIV Incidence in Brazilian Transgender Women
Carolina Coutinho, Emila M. Jalia, Eduardo M. Peixoto, Laylla Monteiro, Bianca Fernandes, Mayara Secco Torres da Silva, Monica D. Pedrosa, Cristiano R. Castro, Marcos D. Sousa, Ruth K. Friedman, Ronaldo Moreira, Sandra W. Cardoso, Valdieca Veloso, Beatriz Grinztejn
Oswaldo Cruz Foundation – Fiocruz, Rio de Janeiro, Brazil

**Background:** Transgender women (TGW) are disproportionately affected by HIV in Brazil. Although HIV pre-exposure prophylaxis (PrEP) is available to vulnerable populations through the Public Health System, its uptake and
1022 HIV Situation in Areas Surrounding Larkana: Findings From Community Test and Treat Implementation

Muhammad S. Jamal1, Muhammad S. Pasha2, Tanveer Hussain3, Shahida Meman2, Atif Ali4, Alfat A. Soomro3, Saima Mushtaq2, Sikander Meman2, Joumana Hermez3

1World Health Organization Regional Office for Eastern Mediterranean, Cotonou, Benin, West Africa Health Organization Country Office for Pakistan, Islamabad, Pakistan, HIV Treatment and Support Centre Rатodero, Larkana, Pakistan, 2Bridge Consultants Foundation, Karachi, Pakistan, Community-Directed Interventions (CDI) Control (HIV-AIDS), Karachi, Pakistan

Background: An outbreak of HIV among children was reported in Ratodero (district Larkana, Sindh province, Pakistan) in April 2019, with unsafe injections and infusions in healthcare settings as the primary mode of transmission. Anecdotal evidence suggests high HIV burden in surrounding districts of Larkana. To date, no data are available on HIV positivity or burden in these areas. We report the results of a first community-based educate, test and treat implementation to understand the HIV situation in surrounding areas of Larkana.

Methods: Community-based door-to-door testing was performed in partnership with CDC Sindh and local administration. Testing focused on two tehsils neighboring Larkana, one in district Jacobabad (159,019 population) and one in district Shikarpur (316,513), for which routine data and expert opinion suggested high burden of undiagnosed HIV. Those aged 18 months to 60 years were among children (18-24 years), earning less than one minimal wage/month, and four years or less of schooling were associated with increased risk of HIV seroconversion.

Results: Between August 2015 and December 2022, 255 HIV-negative children were enrolled with at least one follow-up visit, contributing to 355.37 person-years. The median age was 29 years (interquartile range: 24-38). Overall, 25 TGW seroconverted for HIV during follow-up (HIV incidence: 3.50%, 95% confidence interval: 2.30-5.06). The HIV incidence was higher among younger children (18-24 years: 7.87% [95% CI: 1.71-3.67]) vs. >24 years: 2.19% [95% CI: 1.17-3.67]) and those who have never used PrEP (10.68% [95% CI: 6.47-15.92%] vs. 0.77% [95% CI: 0.24-1.80]) among those who never used to use it. On multivariate Cox models, ever using PrEP was associated with a lower risk of HIV seroconversion, while younger age (18-24 years), earning less than one minimal wage/month, and four years or less of schooling were associated with increased risk of HIV seroconversion.

Conclusion: Despite the availability of prevention strategies provided by the Brazilian public health system targeting key populations, HIV incidence remains high among TGW in Brazil, especially among the younger, less educated, and disenfranchised. As the HIV response continues to fail the most vulnerable individuals, an urgent call to effectively reach this population at risk and address inequalities becomes an imperative need.

Table 1. Risk factors to HIV seroconversion among TGW enrolled on the Transcendendo cohort, Rio de Janeiro, Brazil, 2015-2023.

<table>
<thead>
<tr>
<th>Variable</th>
<th>RRR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever used PrEP [Yes]</td>
<td>0.01</td>
<td>0.00-0.08 &lt;0.001</td>
</tr>
<tr>
<td>Age &gt; 24 years [Yes]</td>
<td>0.70</td>
<td>0.25-1.94 0.001</td>
</tr>
<tr>
<td>Income of &lt;1 minimal wage/ month [Yes]</td>
<td>0.78</td>
<td>0.25-2.62 &lt;0.001</td>
</tr>
<tr>
<td>Schooling equal to 4 years or less [Yes]</td>
<td>10.34</td>
<td>2.63-40.62 &lt;0.001</td>
</tr>
</tbody>
</table>

1CI: confidence interval, RRR: relative risk, TGW: transgender women
Rapidly Changing Socio-Economic Patterns of HIV Incidence in Rural KwaZulu-Natal, South Africa

Paul Mee 1, Elphas Okango 2, Hao-Young Kim 3, Adrian Dobra 4, Khai Hoan Tram 5, Dickman Gareta 6, Kobus Herbst 7, Frank Tanser 8

1 University of Lincoln, Lincoln, United Kingdom, 2 Africa Health Research Institute, Mbabane, Eswatini, 3 South Africa, 4 New York University, New York, NY, USA, 5 University of Washington, Seattle, WA, USA, 6 Stellenbosch University, Stellenbosch, South Africa

Background: Despite a decline in HIV incidence in South Africa associated with the increased provision of HIV prevention services, there remains a lack of evidence on whether this progress has been experienced equitably by those most economically disadvantaged. We assessed whether the risk of acquiring HIV has changed over time for those in different socio-economic strata.

Methods: The study used data from a population-based HIV testing platform run by the Africa Health Research Institute (AHRI) in KwaZulu-Natal, South Africa. Socio-economic status was derived using a Principal Components Analysis, with input variables representing asset ownership and services used. The households were stratified into three equal wealth quantiles in each year. Time to seroconversion was defined as the time between the dates of the last negative and positive HIV tests. Kaplan-Meier curves stratified by socio-economic strata were constructed using two open cohorts. For period 1 (2005 to 2014) the criteria for ART initiation was CD4 count <= 350 cells/ml until (2005 to 2014) the criteria for ART initiation was CD4 count <= 350 cells/ml until 2016 and then ART initiation at any CD4 count starting in 2017. The p-value of a Mantel-Haenszel (MH) test was used to assess whether the trajectories of the curves differed significantly. A Cox PH model was developed to test statistical significance controlling for other covariates.

Results: During the 2005 to 2014 period (N=18,236) there were 2521 seroconversion events over 75086 person-years (PY) of observation (Fig A). Those in the least wealthy socio-economic strata had a higher rate of seroconversion (MH p-value <= 0.001) and a higher incidence than those in the wealthiest strata (4.19/100 PY vs 3.13/100 PY). During the 2015 to 2022 period (N= 14594), there were 901 seroconversions recorded over 51485 PY (Fig B). The wealth trend had reversed with the least wealthy having a lower rate of seroconversion (MH p-value <= 0.001) and a lower incidence than those in the medium and wealthiest strata (1.37/100 PY vs 1.76/100 PY and 2.12/100 PY respectively). A Cox PH multivariate analysis controlling for age and sex used. The households were stratified into three equal wealth quantiles in each year.

Conclusion: This study provides clear evidence that dramatic changes have occurred over time in the association between wealth status and the risk of acquiring HIV, with those in the lowest wealth strata having the lowest seroconversion rate since the move towards universal ART.

Ultra-High Resolution GPS to Measure Human Mobility in High HIV Prevalence Areas in Rural S. Africa

Khai Hoan Tram 1, Elphas Okango 2, Thulile Mathenjiwa 3, Paul Mee 4, Hao-Young Kim 5, Till Bannighausen 6, Adrian Dobra 7, Frank Tanser 8

1 University of Washington, Seattle, WA, USA, 2 Africa Health Research Institute, Mbabane, Eswatini, 3 South Africa, 4 Lincoln International Institute of Rural Health, Lincoln, United Kingdom, 5 New York University Grossman School of Medicine, New York, NY, USA, 6 Heidelberg University, Heidelberg, Germany, 7 Stellenbosch University, Cape Town, South Africa

Background: Mobility, including both short-term travel and migration, has been linked to increased risk of HIV infection. Ultra-high resolution GPS data collected from study participants who carry smartphones during their daily activities offers new insights into the mobility patterns of a representative sample of young adults in an HIV hyper-endemic setting in rural KwaZulu-Natal, South Africa.

Methods: This study involved a random sample of adults aged 20-30 years who were consented and tested for HIV in the 2019 Africa Health Research Institute survey. Between 6/2021 – 3/2023, participants either received a smartphone with a customized Ethica mobile app or installed the app on their own devices. Positional data were recorded at a frequency of up to ~1 second (depending on internet connectivity and movement) over a six-month period. Data was analyzed to explore space-time dimensions of mobility and to quantify time spent in known high HIV prevalence areas. In this analysis, the cluster where each individual recorded the most time spent was considered to be their residential cluster.

Results: In total, 27,151,329 time-stamped location records were collected for 202 individuals (median 74,864.5 data points), spatially distributed across 45 clusters within the study area and 57 municipalities across South Africa. Participants recorded a median of 129.7 days (IQR 71.8-161.8) inside the study area versus a median of 17.2 days (IQR 3.0-48.1) outside the study area. Nearly all (97.0%) made at least one trip outside the study area during this time. Similar proportions spent greater than 50% of their time outside their own residential cluster: either in other clusters within the study area (74.0%) or outside the study area entirely (26.0%). Over half (68/157, 56.1%) of those participants who did not reside in a geographical HIV cluster spent time in those areas. Median percent of recorded time by non-residents in the HIV clusters was 0.6% (IQR 0.04-5.8).

Conclusion: Young adults in rural KwaZulu-Natal display highly dynamic patterns of mobility both locally and farther afield. Over forty percent of this population moved through clusters in the study area with known high HIV prevalence but did not reside in those places. Determining typologies of movement in this age group and quantifying movements in and out of known HIV hotspots can facilitate designing location-intelligent, real-time precision interventions for those at high risk of HIV acquisition.

Centers for Disease Control and Prevention, Kampala, Uganda, 3 Johns Hopkins University School of Public Health, Baltimore, MD, USA, 4 Yale Health Sciences Program, Kigali, Rwanda, 5 National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

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HIV Seroprevalence, Incidence, and Viral Suppression Among Ugandan Female Barworkers

Xinyi Feng 1, Slisha Shrestha 2, Fred Nalugoda 3, Godfrey Kigozi 4, Robert Siekhubu 1, Larry W. Chang 5, Andrea Wirtz 6, Caitlin E. Kennedy 7, Arthur G. Fitzmaurice 8, Gertrude Nakigozi 9, Aaron A. R. Tobian 10, Steven J. Reynolds 11, Joseph Kagaayi 12, Mary Kate Grabowska 13

1 The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2 The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 3 Yale Health Sciences Program, Kigali, Rwanda, 4 Centers for Disease Control and Prevention, Kampala, Uganda, 5 National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

The study used data from a population-based HIV testing platform run by the Africa Health Research Institute (AHRI) in KwaZulu-Natal, South Africa. Socio-economic status was derived using a Principal Components Analysis, with input variables representing asset ownership and services used. The households were stratified into three equal wealth quantiles in each year. Time to seroconversion was defined as the time between the dates of the last negative and positive HIV tests. Kaplan-Meier curves stratified by socio-economic strata were constructed using two open cohorts. For period 1 (2005 to 2014) the criteria for ART initiation was CD4 count <= 350 cells/ml until (2005 to 2014) the criteria for ART initiation was CD4 count <= 350 cells/ml until 2016 and then ART initiation at any CD4 count starting in 2017. The p-value of a Mantel-Haenszel (MH) test was used to assess whether the trajectories of the curves differed significantly. A Cox PH model was developed to test statistical significance controlling for other covariates.

Results: During the 2005 to 2014 period (N=18,236) there were 2521 seroconversion events over 75086 person-years (PY) of observation (Fig A). Those in the least wealthy socio-economic strata had a higher rate of seroconversion (MH p-value <= 0.001) and a higher incidence than those in the wealthiest strata (4.19/100 PY vs 3.13/100 PY). During the 2015 to 2022 period (N= 14594), there were 901 seroconversions recorded over 51485 PY (Fig B). The wealth trend had reversed with the least wealthy having a lower rate of seroconversion (MH p-value <= 0.001) and a lower incidence than those in the medium and wealthiest strata (1.37/100 PY vs 1.76/100 PY and 2.12/100 PY respectively). A Cox PH multivariate analysis controlling for age and sex confirmed these findings.

Conclusion: This study provides clear evidence that dramatic changes have occurred over time in the association between wealth status and the risk of acquiring HIV, with those in the lowest wealth strata having the lowest seroconversion rate since the move towards universal ART.
Background: Female bar workers (FBW) in eastern Africa often engage in sex work. However, population-level data on HIV seroprevalence, incidence, and viral suppression among FBW are rare.

Methods: FBW were identified through longitudinal population-based HIV surveillance in southern Uganda via the Rakai Community Cohort Study between 2012 and 2019. Surveillance was conducted across five surveys in four high HIV prevalence (>35%) Lake Victoria fishing communities and 36 moderate HIV prevalence (~12%) inland agrarian and trading communities. Participants were classified as FBW if they reported bar work as a primary or secondary occupation. Sociodemographics and sexual behaviors of FBW were compared with participants never classified as FBW (non-FBW). Primary outcomes included laboratory-confirmed HIV seroreversion, seroincident HIV infection measured via paired serologic testing, and HIV viral load suppression (<200 copies/ml). Prevalence and incidence rate ratios (PR, IRR) were estimated using age-adjusted Poisson regression models with 95% confidence intervals (95%CI).

Results: A total of 23,556 female participants contributed 52,708 person-visits. Overall, 1,205 (5.1%) women self-identified as FBW at one or more study visits. FBW were significantly older, more likely to report recent migration, and had substantially higher levels of HIV-associated risk behaviors, including higher numbers of lifetime and prior-year sexual partners and alcohol use before sex, compared to non-FBW. FBW also had significantly higher HIV seroprevalence irrespective of age or community (Figure). Among 364 FBW participating in at least four surveys, only 50 (13.7%) self-reported being a bar worker at all surveys, while 128 (35.2%) reported bar work at only a single survey. Overall, 377 HIV incident events occurred over 41,030 years of participant follow-up. Incidence among FBW was 1.76/100 person-years versus 0.90/100 person-years among non-FBW (adjIRR: 2.24; 95%CI: 1.27-3.63). However, FBW living with HIV irrespective of antiretroviral use tended to have higher levels of population HIV viral load suppression compared to non-FBW (62.9% vs 54.9%; adjPR: 1.08, 95%CI: 1.01-1.37).

Conclusion: Enhanced prevention services for female bar workers in Uganda are needed and may reduce HIV incidence in this population. However, high mobility and frequent fluctuation in and out of bar work may complicate delivery of interventions, such as long-acting injectable pre-exposure prophylaxis.
(n=7,206; 35%) and half of men (n=6,216; 52.9%) reported consistent condom use with these partners. Both male and female self-reported never-users were more likely to be older and in non-marital monogamous relationships of significantly longer duration. HIV incidence rates were similar among women who reported never using condoms (0.9/100 pys; 95% CI: 1.39–1.93), always using condoms (0.9/100 pys, 95% CI: 0.64–1.16), and men reporting inconsistent use (1.2/100 pys, 95% CI: 0.88–1.60). Among women, incidence was lowest among those reporting never using condoms (1.4/100 pys; 95% CI: 0.76–2.56) followed by always-users (2.0/100 pys; 95% CI: 1.40–2.91), and highest among inconsistent users (2.8/100 pys; 95% CI: 2.17–3.65).

Conclusion: In this study, self-reported never-users had lower or similar incidence compared to self-reported always-users, suggesting self-reported condom use is not a robust indicator of HIV risk. Reviewing self-reported condom use as a PrEP eligibility criterion might improve PrEP accessibility for those with increased HIV risk.

1029 An HIV-1 Risk Assessment Tool for Women in 15 African Countries: A Machine Learning Approach

Nora E. Rosenberg, Bennie E. Shook-Sa, Abimer M. Young, Yating Zou, Lynda Stanisich-Chibanda, Marcel Yotiebingi, Nadia Sam-Agudu, Sam Phiri, Linda-Gail Bekker, Sizulu Moye, Manhattan Charurat, Jessica E. Justman, Michael Hudgens, Benjamin H. Chi

University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, University of Zimbabwe, Harare, Zimbabwe, Albert Einstein College of Medicine, Bronx, NY, USA, University of Minnesota, Minneapolis, MN, USA, Partners in Hope, Lilongwe, Malawi, University of Cape Town, Cape Town, South Africa, Human Sciences Research Council, Pretoria, South Africa, University of Maryland, Baltimore, MD, USA, Columbia University, New York, NY, USA

Background: Women in Africa disproportionately acquire HIV-1. Understanding which women are most likely to acquire HIV-1 can guide focused prevention, including promotion of pre-exposure prophylaxis (PrEP). We used machine learning to develop a risk assessment tool to identify women most likely to acquire HIV-1 across African countries and to estimate HIV-1 infections averted with focused PrEP.

Methods: Nationally representative data were collected from 2015–2019 from 15 population-based household surveys. This analysis included women aged 15–49 years who tested HIV-1 sero-negative or had recent HIV-1, characterized by HIV limiting antibody avidity enzyme immunoassay, HIV-1 viral load, and detection of antiretroviral drugs in their survey blood samples. Least absolute shrinkage and selection operator regression models were fit with 28 variables to predict recent HIV-1. Models were trained on the full population and internally validated using five-fold cross validation. Performance was evaluated using area under the receiver-operating-characteristic curve (AUC). Sensitivity, specificity, and potential cases averted were estimated, assuming perfect PrEP adherence among all women at key HIV-incidence thresholds.

Results: Among 209,012 participants 248 had recent HIV-1 infection, representing 118 million women and 402,000 (95% CI: 309,000–495,000) new infections annually. Only two variables were retained: living in a subnational area representing 118 million women and 402,000 (95% CI: 309,000–495,000) new annual infections. Only two variables were retained: living in a subnational area representing 118 million women and 402,000 (95% CI: 309,000–495,000) new infections annually. Only two variables were retained: living in a subnational area representing 118 million women and 402,000 (95% CI: 309,000–495,000) new annual infections.

Conclusion: HIV-1 acquisition is not evenly distributed, with two thirds of infections occurring among a small fraction of women. This predictive, generalizable, and parsimonious tool has the potential to guide high-impact PrEP delivery.

1030 Recent HIV Infections and HIV Incidence in PrEP Among Adolescents From Key Populations in Brazil

Diana Reyna Zeballos Rivas, Fabiane Soares, Lailo Magno, Célia Landmann, Orlando Ferreira, Matheus Westin1, Direo Greco, Alexandre Granger1, Ines Dourado1 for the PrEP1519 Study Group

1Federal University of Bahia, Salvador, Brazil, 2State University of Bahia, Salvador, Brazil, 3Usovaldo Cruz Foundation - Focruz, Rio de Janeiro, Brazil, 4Universidade Federal de Rio de Janeiro, Rio de Janeiro, Brazil, 5Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, 6University of Sao Paulo, Sao Paulo, Brazil

Background: Over the last decade, HIV infection has increased among young men in Brazil, and PrEP is an effective prevention method that can help to reduce HIV incidence in this population. Recent HIV-1 infection (RHI) testing algorithms (RITAs) are used to estimate HIV incidence from cross-sectional data. We aimed to assess RHI among adolescents from key populations at enrollment and HIV incidence among adolescent men who have sex with men (aMSM) and transgender women (aTGW) who started using daily oral PrEP.

Methods: PrEP1519 study is a PrEP demonstration cohort in three Brazilian capital cities among sexually active aMSM/aTGW aged 15–19. We conducted a cross-sectional analysis with baseline data from 2020 including participants who were screened with a fourth-generation HIV rapid test. The RITA tested blood specimens using Sedia LAg assay that detects antigen-driven antibody response (Sedia BioSciences, Portland, OR), an HIV-1 RNA test, viral load and a CD4 count prior to ART use. Among those participating, RITA-based HIV incidence was calculated using a mean duration of recession infection of 167 days and a false-negative rate of 0.02. We included adolescents who had a HIV negative test and initiated PrEP in 2020 were used to calculate HIV incidence on PrEP. Incidence was calculated considering the time from first PrEP prescription until the last HIV test, PrEP prescription, or visit in 2020. Tenofovir-diphosphate (TDF-DP) concentrations in dried blood spots were estimated for those who tested positive for HIV.

Results: Out of the 494 participants screened, 21 tested positive. Among those, 6 were classified as recent infections and 15 as long-term infection. At baseline, the HIV prevalence was 4.3%, and the estimated RHI was 2.6 (95% CI 0.5–4.8) per 100 person-years. The HIV incidence over 418 adolescents who started PrEP was 2.5 (95% CI 0.7–6.4) per 100 person-years. All the adolescents who seroconverted had levels of TDF-DP below limits of quantification.

Conclusion: The estimated RHI rate highlights the need for targeted HIV prevention interventions for sexual minorities adolescents. Integrating RHI testing into routine can aid in monitoring the effectiveness of prevention efforts and improve early entry to HIV care. Low adherence and PrEP discontinuation are challenges that need to be addressed among adolescents to enhance PrEP effectiveness. One strategy to tackle these issues involves offering adolescents a range of choices, including long-acting PrEP.

1031 HIV Incidence and Factors Associated With Never Testing for HIV Among Young MSM: Conectad@s Study

Sylvia L. Teixeira1, Emilía M. Jalil2, Cristina M. Jalil2, Rodrigo Scarparo, Valdilea Veloso2, Erin Wilson3, Willi McFarland4, Beatriz Grinsztejn5, Thiago S. Torres5, for the Conectad@s Study Team

1Usovaldo Cruz Institute, Rio de Janeiro, Brazil, 2Instituto Nacional de Infectologia Encontro Chagas, Rio de Janeiro, Brazil, 3San Francisco Department of Public Health, San Francisco, CA, USA

Background: New HIV cases are increasing among young men who have sex with men (YMSM) in Brazil. Research is needed to monitor new infections and examine HIV prevention engagement, including HIV testing. For this study, we estimated HIV incidence and explored HIV testing and factors associated with never testing for HIV in a large study of YMSM in Brazil.

Methods: Data are from the Conectad@s study of YMSM aged 18–24 years in Rio de Janeiro, Brazil. Participants were recruited between Nov/2021-Oct/2022 using respondent driven sampling. Participants were offered HIV testing and were surveyed on demographics, behavior, sexually transmitted infection (STI) and HIV knowledge (validated 12-items measure with scores ranging from 0 to 12; higher scores mean higher knowledge). Recent HIV infection was determined using the Maximi HIV-1 LAg-Avidity EIA assay as part of recent infection testing algorithm (RITA). Annualized HIV incidence and 95% confidence intervals (95% CI) were calculated using the UNAIDS/WHO incidence estimator tool, excluding participants with known HIV diagnosis and those currently on PrEP. We used logistic regression models to assess factors associated with never testing for HIV.
Declines in HIV Incidence Among Gay, Bisexual and Other MSM in Victoria, Canada, 2012–2017–23

David Moore1, Lu Wang, Allan Lal, Justin Barath, Julie S. Montaner, Mark Hall, Junine Toy, Viviane Dias Lima, Paul Sereda, Robert Hogg, Nathan Lachowsky

Background: British Columbia (BC), Canada, has provided dedicated funding to support expanded access to HIV testing and engagement in HIV care since 2010 and publicly funded PrEP since 2018. We compared HIV incidence for gay, bisexual and other men who have sex with men (GBM) in metropolitan Vancouver over an 11-year period.

Methods: Participants were sexually active GBM aged ≥16 years and were recruited through respondent driven sampling into two cohort studies: Momentum I (M1; 2012–2019) and Momentum II (M2; 2017–2023). Participants completed a self-administered computer-based survey and tests for HIV and other sexually transmitted infections every 6 months. For participants with HIV negative at enrollment, we measured HIV incidence over four years of follow-up through data linkages with the BC HIV Drug Treatment Program. We excluded participants from the M2 analysis who also participated in M1. We calculated HIV incidence rates and rate ratios across time periods and used Poisson regression to identify risk factors for HIV infection, stratified by time period.

Results: We recruited 774 participants in M1 and 753 in M2. Of these, 551 in M1 and 533 in M2 were HIV-negative at enrolment and included in the analysis. HIV PrEP use was reported by a maximum of 6.3% of participants at any visit in M1 and increased from 6.4% from 2017 to 59.6% in 2023 for M2 participants. We identified 16 new HIV infections in 2166.51 person-years (PYRs) of follow-up for an incidence rate (IR) of 0.74 per 100 PYRs in M1; and 6 new infections in 2166.51 PYRs for an IR of 0.28 per 100 PYRs in M2 (IRR = 0.38; 95% CI: 0.15 – 0.97). In M1, HIV incidence was associated with age <30 years (IRR 2.74; p=0.056), having an incidence rate [IR] of 0.74 per 100 PYRs in M1; and 6 new infections in 2166.51 person-years (PYRs) of follow-up for 16 new HIV infections in 2166.51 person-years (PYRs) of follow-up for 16 new HIV infections in 2166.51 person-years (PYRs). We enrolled 400 cisgender YMSM; median age was 21 years (IQR: 19-23), 166 (41.6%) self-identified as Black, 241 (60.2%) completed secondary education, and 3 (0.8%) were currently using PrEP. Forty participants tested positive for HIV (HIV prevalence: 10%), 5.0% (N=20/400) newly tested positive for HIV, and of those, 5 (25.0%) had a recent HIV infection. The annualized HIV incidence rate was 2.81% (95%CI: 0.32 – 5.30). Overall, 108 (27.0%) never tested for HIV. Lower HIV knowledge was associated with never testing for HIV. Never testing was associated with being aged 18–19 years, reporting no STI in the last 12 months, fewer sexual partners and no chemsex (Table).

Conclusion: HIV prevalence and incidence among YMSM in our sample was high. Almost a third of YMSM in our study had never been tested for HIV, although all participants accepted testing when offered. These results point to an urgent need for focused policies and interventions to reach and improve HIV prevention engagement among younger MSM in Brazil. Strategies to raise knowledge and awareness of the need to get tested for HIV among parents and in schools and among youth may be needed to create demand for HIV prevention methods such as testing and PrEP that are readily available in Brazil.

Table: Factors associated with never testing for HIV among young men who have sex with men (YMSM) in Rio de Janeiro, Brazil, 2011–2012.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N=108</th>
<th>Never Tested (N=108)</th>
<th>Ever Tested (N=569)</th>
<th>OR (95%CI)</th>
<th>Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of male sex partners in P6M</td>
<td>N=108</td>
<td>11.13 (9.25 – 13.01)</td>
<td>2.74 (0.32 – 5.30)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.74</td>
</tr>
<tr>
<td>CAS with an HIV-positive or unknown serostatus partner</td>
<td>N=108</td>
<td>11.38 (9.26 – 13.50)</td>
<td>2.74 (0.32 – 5.30)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.74</td>
</tr>
<tr>
<td>Crystal meth use in P6M</td>
<td>N=108</td>
<td>6.44 (0.07 – 0.17)</td>
<td>3.75 (1.02 – 12.93)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>HIV incidence in M1</td>
<td>N=108</td>
<td>11.38 (9.26 – 13.50)</td>
<td>2.74 (0.32 – 5.30)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.74</td>
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**1032 Factors Associated With Low-Level Viremia in People Living With HIV: 9-Year Retrospective Study**

Eunmi Yang, Doh Hee Kim, Subin Kim, Mi Young Ahn, Dong Hyun Oh, Jae-Phil Choi

Seoul Medical Center, Seoul, Korea, Republic of Korea

Background: The goal of antiretroviral therapy (ART) is to sustain the suppression of human immunodeficiency virus (HIV) viral load. The prognostic value and clinical outcomes of low-level viremia (LLV) remain unclear. The goal of this study was to investigate the risk factors for LLV and their association with virologic rebound in Korea.

Methods: We retrospectively reviewed the records of all patients registered with HIV infection at Seoul Medical Center, South Korea from January 2014 to December 2022. Patients starting ART and achieving viral suppression were included in the study. LLV was defined as at least 2 consecutive viral loads ≥40,000 copies/mL. We analyzed the association between the clinical factors and LLV using multivariable logistic regression.

Results: During the study period, 382 patients were enrolled and 13 (3.4%) patients experienced LLV. Compared to controls, LLV patients have higher incidence of diabetes mellitus than control patients (p = 0.04). Age, sex, Charlson comorbidity index, ART regimen, initial acquired immune deficiency syndrome (AIDS) defining conditions, and resistance-associated mutation did not differ between the two groups. During the study period, there was no virologic rebound, new onset AIDS-defining illness, or death in LLV patients. We analyzed a multivariable logistic regression model that incorporated age,
diabetes mellitus, and resistance-associated mutation. Age was an independent risk factor for LLV (aOR 1.07; 95% CI 1.01–1.12).

**Conclusion:** In this cohort, LLV was not associated with AIDS, virologic rebound, and death. The age at which patients achieve viral load suppression may be a risk factor for LLV. Further research with larger cohorts is warranted to evaluate the risk factors and outcomes of LLV.

<table>
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<tr>
<th>Incidence of Viral Rebound and Associated Risk Factors Among Adults Living With HIV in Tanzania</th>
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</table>
| **Methods:** This was a retrospective cohort study, and we extracted 100,052 records of new PLHIV from the national care and treatment (CT) database on ART in 2019 and reviewed client-level data for 3.6 years. A standard Cox model was used to determine hazard ratios (HR) for viral rebound across different demographic and clinical characteristics. An adjusted model was developed on variables that were significant in univariate analysis. The results of the regression analysis present HR and their corresponding 95% confidence intervals. All analyses were conducted using STATA Version 18.

**Results:** The overall viral rebound rate was 152.0 (95% CI: 144.7-157.7) per 1,000 person-years. The rates were higher in Dar es Salaam (178.0; 95% CI: 165.0-192.1) and Tabora (177.9; 95% CI: 163.2-194.2) followed by Kagera (137.1; 95% CI: 125.5-149.8) and Geita (116.4; 95% CI: 107.2-126.2) per 1,000 person-years. Among patients with poor adherence to antiretroviral therapy (ART), viral rebound was 209.43; (95%CI: 167.3-263.9), and with good adherence, viral rebound was 151.25; 95% CI:144.0-158.9). In the adjusted analysis, the following factors were found to be associated with viral rebound: WHO classification Stage IV at diagnosis (adjusted hazard ratio (aHR) 1.07, 95% CI 0.94-1.22); Not changing ART regimen (aHR 2.10, 95% CI 1.95-2.25); Poor ART adherence (aHR 1.49, 95% CI 1.22 -1.82); Female sex (aHR 1.49, 95% CI 1.22 -1.82). The results were similar when compared with VL suppression. In the adjusted analysis, the following factors were found to be associated with viral rebound: WHO classification Stage IV at diagnosis (aHR 1.07, 95% CI 0.94-1.22); Not changing ART regimen (aHR 2.10, 95% CI 1.95-2.25); Poor ART adherence (aHR 1.49, 95% CI 1.22 -1.82); Female sex (aHR 1.49, 95% CI 1.22 -1.82). The results were similar when compared with VL suppression.

**Conclusion:** Any detectable VL is a risk for virological failure. This requires strategies that support and strengthen stepped-up adherence counselling for all clients with a detectable VL to limit progression to virological failure and for contextualized care for males and PLHIV aged <35 years.

**1037 HIV Data Informed Care: Using Routine Data to Identify Persons At Risk of Developing Viremia**

**Thomas Martin**, Ravi Goyal, Gordon Honerkamp Smith, Alan Wells, Samantha Tweeten, Diana Corona-Mata, Susan J. Little

**University of California San Diego, San Diego, CA, USA,** **University of California San Diego, La Jolla, CA, USA,** **San Diego Department of Public Health, San Diego, CA, USA,** **Hospital Universitario R Neck Soria, Cordoba, Spain**

**Background:** Identifying persons with HIV (PWH) at increased risk of developing unsuppressed viral load or falling out of care could lead to improved resource allocation and improve the focus of public health data-to-care activities. We sought to evaluate factors associated with developing unsuppressed viral load in San Diego County, California using routinely reported HIV data.

**Methods:** Data were obtained from the Enhanced HIV/AIDS Reporting System (eHARS) for PWH who were diagnosed or resided in San Diego County. The analysis was limited to individuals whose diagnosis date was known and occurred after May 2017 (based on eHARS data availability) and had attained viral suppression (VL<200 copies/mL after attaining viral suppression). Survival curves for each group were estimated separately using the Kaplan-Meier estimator and compared using log-rank tests. To compare viral load testing patterns between the rebound and no rebound group, we used Wilcoxon rank sum tests.

**Results:** Between June 2017 and December 2021, 1840 persons were diagnosed with HIV and achieved viral suppression. From these participants there was a total of 12,222 viral load measurements included in the analysis. Among these individuals, 244 (13.3%) subsequently developed an unsuppressed viral load (>200 copies/mL). Factors associated with an increased rate of unsuppressed
viremia included younger age at diagnosis (p=0.013), Black race or Hispanic ethnicity (p=0.04), female birth sex (p=0.04), persons with any history of injection drug use (IDU, p=0.003), men who have sex with men (MSM) who also report IDU, and a later HIV stage at diagnosis (p<0.001). Viral load testing pattern analysis indicated that among persons with at least 1 year of follow-up, less frequent testing (p<0.001) and lower variance in testing interval (p=0.002) were associated with developing unsuppressed viral load.

Conclusion: Multiple demographic, HIV associated factors, and viral load testing patterns were associated with an increased risk of developing unsuppressed HIV viral load. These variables may be considered as part of a risk score to identify individuals before they develop unsuppressed viremia. Prospective evaluation is needed to determine if such a score could lead to greater clinical and social support services to prevent viral load rebound. The figure, table, or graphic for this abstract has been removed.

1038 Longitudinal Viral Load Clustering for People With HIV Using Functional Principal Component Analysis

Jiayang Xiao, Yunqing Ma, Xueying Yang, Bankole Olatosi, Xiaoeming Li, Jiaya Zhang
University of South Carolina at Columbia, Columbia, SC, USA

Background: While multiple indicators like viral suppression (VS) and viral rebound (VR) exist for monitoring HIV viral load (VL), research on continuous VL clustering is limited. By characterizing people with HIV (PWH) into distinct groups, stratified long-term risks of virological failure can be assessed. Therefore, this study aims to use functional data clustering to identify continuous VL patterns and characterize each cluster by demographics, comorbidities, social behaviors, CD4 count, and antiretroviral therapy (ART) history.

Methods: We analyzed adult PWH diagnosed from 2005 to 2020 in South Carolina with a 5-year minimum follow-up from the first VS to the last VL test. Functional principal component analysis (FPCA) was used to categorize PWH into clusters based on sparse VL test. ANOVA were used to test the difference in VL characteristics, demographics, comorbidities, social behaviors, and longitudinal CD4 count during the follow-up period of each cluster. Subgroup analyses were conducted among PWH with ART, examining the ART patterns within each cluster, including initial, most recent, most frequently used ART, and any regimen switches.

Results: A total of 5,916 PWH were grouped into four clusters: long-term VS group (Cluster 1, 17.3%), short-term VS group (Cluster 2, 29.8%), suboptimal VS group (Cluster 3, 23.8%), and viral failure group (Cluster 4, 24.9%). In Cluster 1 with an average of 11-year follow-up, PWH displayed sustained VS (95.3%), lower mean CD4 count (28.1%), most NRVT+NNRTI in first and last 3 months (72.8% and 64.0%), and less regimen switches (32.0%). Results for Cluster 2 were similar except for shorter follow-up (6 years), more comorbidities (31.4%), and higher max CD4 count (48.4%). In Cluster 3, PWH were mostly under 30 years old (44.8%) and Black (77.2%), with relatively lower mean VL (92.9%), lower number of VR (18.4%), higher maximum CD4 count (47.6%), and were similar except for shorter follow-up (6 years), more comorbidities (31.4%), and higher max CD4 count (48.4%). In Cluster 4, demographics were similar to Cluster 3, while PWH had higher mean VL (40.6%), lower mean CD4 count (31.4%), received most PI+NNRTI (32.4%) in the first 3 months, and switched the regimen more frequently (55.2%).

Conclusion: The findings highlight the value of continuous clustering in understanding the distinct viral profiles of PWH. By identifying distinct clusters varied in VL patterns, demographics, substance use, comorbidities, CD4 count, and ART, we emphasize the importance of tailored treatment and insights for targeted interventions.

1039 Progress Toward 95-95-95 and Viral Suppression Among FSW/CSEG in Unguja, Zanzibar, 2023

Mtoro J. Mtoro1, Farhat J. Khalid2, Christen A. Said3, Thomas W. John3, Joel Ndayoge1, Sarah Porter1, Mohamed Dahoma1, Tara Pint0, Asha Ussi4, Ahmed Jahzum1, Augustino Msanga5, Pili Khamsi1, Ahmed Khatib1
1Global Programs, Dar es Salaam, United Republic of Tanzania, 2Ministry of Health, Zanzibar, United Republic of Tanzania, 3University of California San Francisco, Dar es Salaam, United Republic of Tanzania, 4US Centers for Disease Control and Prevention Tanzania, Dar es Salaam, United Republic of Tanzania, 5Zanzibar Integrated HIV, Hepatitis, TB, and Leprosy Programme, Zanzibar, United Republic of Tanzania, 6Zanzibar AIDS Commission, Zanzibar, United Republic of Tanzania

Background: An integrated biobehavioral survey (IBBS) in Unguja Island, Zanzibar, in 2019 showed female sex workers and commercially and sexually exploited girls (girls < 18 years given money/ goods for sex) (FSW/CSEG) have a higher HIV prevalence (12.1%) than the general population (0.4%). HIV diagnosis, linkage to antiretroviral treatment (ART), retention, and viral suppression (VS) among FSW/CSEG are critical to epidemic control in Zanzibar. In a follow-up IBBS completed in August 2023, we measured progress towards UNAIDS 95-95-95 goals and VS among FSW/CSEG who completed ≥ 6 months of ART, in Unguja.

Methods: We recruited women aged ≥ 15 years who reported living in Unguja for ≥ 3 months and exchanging sexual intercourse for money in the prior month using respondent-driven sampling. We assessed HIV testing and treatment history through an interviewer-administered questionnaire and offered point-of-care HIV testing in accordance with national guidelines. For these testing HIV-positive, we quantified HIV viral load (VL). We defined VS as < 1,000 HIV RNA copies/mL, which comprises low level viremia (LLV) (50–999 copies/mL) and undetectable VL (< 50 copies/mL). Women who disclosed a positive HIV status or were virally suppressed were categorized as knowing their status. Women who self-reported ART use or were virally suppressed were classified as on ART. We produced weighted point estimates and standard errors, reported as percentages with 95% confidence intervals (CI).

Results: We enrolled 598 FSW/CSEG; median age was 31 years (range: 15–55 years). HIV prevalence was weighted 21% (95% CI: 17–25%) and was highest among women aged ≥ 45 years (47%; 95% CI: 31–62%). Among 138 FSW/CSEG who tested HIV-positive, 92% (95% CI: 85–100%) knew their status, 98% (95% CI: 78–100%) of those who knew their status were on ART, and 88% (95% CI: 79–98%) of those on ART were virally suppressed. Among 84 FSW/CSEG who reported being on ART for ≥ 6 months, 71% (95% CI: 60–82%) had an undetectable VL, 17% (95% CI: 8–25%) had LLV, and 13% (95% CI: 5–21%) were unsuppressed.

Conclusion: HIV prevalence among FSW/CSEG in Unguja remains high. While the UNAIDS target for ART coverage has been met among FSW/CSEG living with HIV, there are still gaps in HIV diagnosis and viral suppression. Finding a detectable VL among FSW/CSEG is critical to epidemic control in Zanzibar.

1040 Individual- and Community-Level Predictors of HIV Care Continuum Progression: Clark County, Nevada

Ravi Goyal1, Alan Wells2, Victoria Burris3, Angel Stachnik3, Preston Nguyen Tang3, Lyell Collins4, Sanjay R. Mehta4, Susan L. Little4
1University of California San Diego, La Jolla, CA, USA, 2Southern Nevada Health District, Las Vegas, NV, USA, 3Nevada Department of Health and Human Services, Carson City, NV, USA, 4VA San Diego Healthcare System, La Jolla, CA, USA

Background: The HIV care continuum provides a comprehensive framework to assess the progress of people with HIV (PWH) from diagnosis to sustained viral suppression.

Methods: We investigated associations between HIV care progression (being diagnosed, being in care, and being virally suppressed) and individual- and community-level characteristics in Clark County, Nevada. Individual-level characteristics included: age at diagnosis, current age, being MSM or IDU, race, sex, education, income, and being HIV genetically clustered (distance threshold < 1.5%). Community-level characteristics included aggregated metrics for education, employment, race, and poverty. We used LASSO (Least Absolute Shrinkage and Selection Operator) regression with a zip code-level random effect to simultaneously conduct model selection and multivariate analyses; model tuning parameter was estimated using cross-validation.

Results: We identified 5,122 diagnosed PWH in Clark County from 2011 to 2022. Of these individuals, 29% were Black, 36% Hispanic, 86% male, 69% men who...
have sex with men (MSM), and 56% with a high school education or less. More recent diagnosis year (estimate -0.14; SE 0.01; p-value: <0.001) and being MSM (est. -0.42, SE: 0.11, p-value: <0.001) were inversely associated with late-stage diagnosis, while older diagnosis age was associated with higher probability (est. 0.04, SE: 0.003, p-value: <0.001); no community-level predictors were associated with late-stage diagnosis. Individual-level predictors associated with being in-care include: MSM (est. 0.31, SE: 0.14, p-value: 0.03), being HIV genetically clustered with an another PWH (est. 0.63, SE: 0.20, p-value: 0.001), more recent diagnosis year (est. 0.15, SE: 0.01, p-value: <0.001), and older age at diagnosis (est. 0.03, SE: 0.004, p-value: <0.001). In addition, residing in areas with higher percentages of poverty (est. -2.67, SE: 1.32, p-value: 0.04) and Hispanics (est. -1.27, SE: 0.65, p-value: 0.050) were significantly associated with being out of care. Similar associations with an individual being in-care were identified for an individual being virally suppressed—though some predictors differed; see Table 1 for details.

Conclusion: Further studies are needed to identify the factors associated with poverty (e.g., access to HIV services) that may contribute to being out of care and virally unsuppressed. This analysis can serve as a basis for proactively identifying and supporting patients at risk of disengaging from HIV care through personalized care plans.

Table 1: LASSO Regression results for associations between HIV care progression (late-stage diagnosis, being in-care, and being virally suppressed) and individual- and community-level characteristics in Clark County, Nevada. Only characteristics that were significant for at least one outcome are shown. Coeffs in red indicate significance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>p-value</th>
</tr>
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<td>Year of diagnosis</td>
<td>-0.14 (0.11)</td>
<td>0.01</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Age of diagnosis</td>
<td>0.04 (0.02)</td>
<td>0.01</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.12 (0.11)</td>
<td>0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Men vs. women with HIV genetic clusters</td>
<td>0.00 (0.02)</td>
<td>0.00</td>
<td>0.96</td>
</tr>
<tr>
<td>Men vs. women with non-Hispanic</td>
<td>0.00 (0.02)</td>
<td>0.00</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Background: While CD4 count trends following ART initiation are well-documented, limited attention has been given to CD4 count evolution after reengagement in care following disengagement from care, especially in cases of multiple disengagements. We aimed to characterize patients prone to disengage from care and compare CD4 trajectories among individuals who never disengaged and those who disengaged once or multiple times.

Methods: Data were obtained from the Athens Multicenter AIDS Cohort Study (AMACS). We included people with HIV (PWH) who initiated ART and were diagnosed after the age of 15. Disengagement was defined as an absence of clinic visits for at least 1.5 years. Multinomial logistic regression was employed to compare the profiles of individuals who have disengaged from care (once or multiple times) with those who have never disengaged. Linear mixed models (LMMs) with subject-specific knots at disengagement and reengagement were used to model CD4 trends on the square-root scale separately for those who disengaged once and twice. Another LMM including individuals who had never disengaged was also fitted for comparison. CD4 evolution conditional on baseline CD4 categories (ART initiation, first and second reengagement) was calculated using properties of the bivariate normal distribution.

Results: 6722 PWH were included, 86% male; 58% MSM and 12% IVDUs. The median age at ART initiation was 36 years. 27% of PWH disengaged from care once and 10% at least twice, with the median time from ART initiation to first and second disengagement being 3.5 and 7 years, respectively. Young IVDUs (<40 years at diagnosis) and particularly those of non-Greek origin were most likely to disengage at least once, with female IVDUs being the most vulnerable. The average gradient of CD4 restoration after ART initiation was the highest among those who never disengaged from care, followed by those who experienced one and 2 gaps in care, whereas the lowest was observed among those who dropped-out of care (Figure A); CD4 restoration after reengagement in care, especially after the second time, was notably worse compared to the corresponding CD4 increase after ART initiation before disengagement, regardless of the baseline CD4 levels (Figure B).

Conclusion: While CD4 counts tend to increase after reengagement in care, it is evident that multiple disengagements cumulatively impact negatively CD4 evolution; young IVDUs and those of non-Greek origin are the most vulnerable for disengagement from care.
1043 Rethinking the Definition of Late HIV Diagnosis Using Florida Surveillance Data, 2015-2021
Christina E. Parisi, Robert Cook, Zhigang Li, Shantrel Candiate, Aweewura Kwara, Zhi Zhou, Natalie Chichetto
University of Florida, Gainesville, FL, USA

**Background:** Late HIV diagnosis is a barrier to ending the epidemic as it may be associated with prolonged transmission risk and worse HIV-related outcomes. The CDC defines late HIV diagnosis as a CD4+ T-cell count <200 cells/mm³ at diagnosis. It is unclear if an expanded clinically relevant definition of CD4<350 would better represent those in need of resources to prevent poor outcomes. We aimed to examine trends in the annual proportion of different definitions (late/delayed) of late HIV diagnosis between 2015-2021 in Florida, characteristics associated with late/delayed diagnosis, and the association between diagnosis status and mortality.

**Methods:** Data included laboratory results for HIV care recipients, diagnosed 2015-2021, in the Enhanced HIV/AIDS Reporting System. Diagnosis status was categorized as late (CD4<200), delayed (200≤CD4<350), and timely (CD4≥350). Characteristics of interest were age, gender, race/ethnicity, birth region, and diagnosis facility. Multivariable multinomial logistic regression models examined characteristics by diagnosis status. Logistic regression, adjusting for characteristics, estimated risk of death during the study period.

**Results:** Of 27,460 individuals (21% female, 39% non-Hispanic [NH] Black, 35% Hispanic, 2% NH Other, mean age 38.3 years [SD 12.9]), 23% had a late and 18% had a delayed diagnosis overall, and this proportion was consistent annually. Older adults (ref=18-24, 25-34 OR 1.6, 95% CI 1.4-1.8), 35-49 [2.4, 2.1-2.7], 50+ [2.7, 2.3-3.0]) and those of Caribbean birth region (ref=North America; 1.1, 1.0-1.3) were more likely to have a late diagnosis. Late and delayed diagnosis were similarly associated with female (m/f: delayed: 0.9, 0.8-0.96, late: 0.8, 0.7-0.9), NH Black (ref=NH White; delayed: 1.6, 1.4-1.7, late: 1.5, 1.4-1.6) Hispanic (delayed: 1.4, 1.2-1.5, late: 1.3, 1.0-1.2), and inpatient diagnosis (ref=outpatient; delayed: 1.6, 1.4-1.7, late: 5.4, 5.0-5.9) characteristics. There were 1,176 deaths and delayed (1,3, 1.0-1.5) and late (1.9, 1.6-2.2) diagnosis were associated with death.

**Conclusion:** Expanding the late diagnosis definition captured an additional 18% of HIV diagnoses that may be at greater risk of poor outcomes such as death. Characteristics associated with late/delayed diagnoses are consistent and both were associated with greater mortality risk. Those with late/delayed diagnosis would benefit from enhanced intervention. Future research can target other late/delayed diagnosis factors to address missed HIV testing opportunities.

1044 Temporal Trends in CD4 Cell Count Soon After Seroconversion and HIV-RNA Viral Set-Point
Nikos Pantzias1, Dominique Costagliola1, Ard van Sighem2, Inna Jarrin†, Laurence Meyer1, Caroline Sabin1, Christina Carlander1, John Gill1, Shema Tarqui†, Bruno Spire1, Fiona Burris1, Elsa Ruiz-Burgas1, Khouloud Porter1, Giota Touloumi1, for the CASCADE Collaboration
1National and Kapodistrian University of Athens, Athens, Greece, 2Katholieke Universiteit Leuven, Leuven, Belgium, 3Stichting HIV Monitoring Foundation, Amsterdam, Netherlands, 4Instituto de Salud Carlos III, Madrid, Spain, 5Université Paris-Sud, Paris, France, 6University College London, London, United Kingdom, 7Karolinska University Hospital, Stockholm, Sweden, 8Southern Alberta Clinic, Calgary, Alberta, 9Aix-Marseille Université, Marseille, France

**Background:** We have previously reported on a temporal decrease in CD4 cell count at seroconversion (SC) and an increase in HIV-RNA viral load (VL) levels at 1 year (which we refer to as set-point) over the period 1980-2008. These markers of virulence reached a plateau in the mid 2000’s. Here we update these analyses, focusing on changes since the introduction of combination ART in 1996.

**Methods:** Data were derived from the CASCADE Collaboration. Included individuals seroconverted ≥1996, were ≥16 years, had an HIV- to- HIV+ test interval ≤1 year or other laboratory evidence of SC, and CD4/ VL measurements while ART naive and AIDS-free. Exploratory analysis revealed gradual changes at ~4 months and ~1 year after SC for CD4 and VL, respectively. Analyses were based on piecewise linear mixed models. Calendar time effects were introduced through natural cubic splines. For CD4 at 4 months after SC and viral set-point analyses, those seroconverting after 1/1/2015 and 1/1/2014, respectively, were excluded, as they tended to initiate ART very soon after SC and reliable estimation of these quantities was not feasible. Models were adjusted for age, sex, transmission mode, region of origin, acute infection (< or ≥30 day HIV test interval) and type of viral assay.

**Results:** Of 28,556 individuals in CASCADE, 15,059 (52.7%) fulfilled the inclusion criteria. The majority (70.5%) acquired HIV through sex between men and 10.3% men and 14.3% women through heterosexual contact. Median (IQR) age at SC was 33 (27, 41) years. Of 2701 with known subtype 2275 (84.2%) had a B subtype. Estimated (95% CI) CD4 cell count levels at 4 months after SC declined from 598 (562-635) to those seroconverting in 1996, to a plateau of ~559 (530-588) cells/µl for those seroconverting between 2006 and 2010 and slowly increased in more recent years (Figure, a). Viral set-point showed a similar but reversed trend with an increase from 4.31 (4.17-4.44) in 1996 to ~4.47 (4.36-4.58) log c/mL in 2006-2010 and a slow declining trend thereafter (Figure, b).

**Conclusion:** Our results showed minimal changes in levels of both CD4 soon after SC and viral set-point after 2004 suggesting that these markers of HIV virulence may have plateaued.

1045 Advanced HIV Disease and the Care Cascade: Trends From 2015-2021
David S. Lawrence1, Melanie Mayo2, Mark W. Tenforde3, Katrina Lechilie4, Charles Muthoga4, Ishepo B. Leeme5, Thomas S. Harrison6, Madisa Mine6, Joseph N. Jarvis7, for the AMBITION Study Group
1London School of Hygiene & Tropical Medicine, London, United Kingdom, 2University of Malawi, Blantyre, Malawi, 3University of Pennsylvania in Botswana, Gaborone, Botswana, 4Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana, 5St George's University of London, London, United Kingdom, 6National Health Laboratory, Gaborone, Botswana

**Background:** The burden of advanced HIV disease (AHD) remains high in much of Africa despite expanded access to ART. Designing interventions to effectively
Global Trends in CD4 Count Measurement and Prevalence of CD4 <200 cells/µL at ART Initiation

Reene De Waal, 1 Kara Wool-Kaloustian, 1 Ellen Brazier, 2 Keri N. Althoff, 2 Antoine Jacquet, 3 Stephany Duda, 4 Nagalingeswaran KumaraSamy, 5 Helen Byakwaga, 6 Gad Murenzi, 7 Amy C. Justice, 8 Didier Kouroum Blouvi, 9 Carina Cesar, 10 Michael J. Silverberg, 11 for International epidemiology Databases to Evaluate AIDS

Background: In this cross-sectional analysis of the Swedish HIV cohort, we identified all people with HIV currently in active care in 2023 from the national register InfCareHIV. We defined five categories of difficult-to-treat HIV: 1) advanced resistance, 2) four-drug regimen, 3) salvage therapy (bitalizumab, fostemsavir, enfuvirtide, maraviroc, etravirine, BID dolutegravir, BID darunavir), 4) virologic failure within the past 12 months, and 5) ≥ 2 regimen switches following virologic failure since 2008. People classified as having difficult-to-treat HIV were compared with non-difficult for background characteristics as well as treatment outcomes (viral suppression [< 50 copies/mL] and self-reported physical and psychological health [based on a validated health questionnaire]) using Pearson’s χ² test as well as logistic regression adjusted for sex, age, and risk group.

Methods: In this cross-sectional analysis of the Swedish HIV cohort, we identified all people with HIV currently in active care in 2023 from the national register InfCareHIV. We defined five categories of difficult-to-treat HIV: 1) advanced resistance, 2) four-drug regimen, 3) salvage therapy (bitalizumab, fostemsavir, enfuvirtide, maraviroc, etravirine, BID dolutegravir, BID darunavir), 4) virologic failure within the past 12 months, and 5) ≥ 2 regimen switches following virologic failure since 2008. People classified as having difficult-to-treat HIV were compared with non-difficult for background characteristics as well as treatment outcomes (viral suppression [< 50 copies/mL] and self-reported physical and psychological health [based on a validated health questionnaire]) using Pearson’s χ² test as well as logistic regression adjusted for sex, age, and risk group.

Results: Nine percent of the Swedish HIV cohort in 2023 met at least one criterion for difficult-to-treat HIV. The most frequent category was ≥ 2 switches following failure (6%), followed by “advanced resistance” (2%) and “salvage therapy” (2%). Compared with non-difficult, people with difficult-to-treat HIV were older, had an earlier first year of positive HIV test and lower CD4+ T-cell counts. Women were overrepresented among people classified as having difficult-to-treat HIV, especially in the categories “recent virologic failure” and “≥ 2 switches following failure”. The viral suppression rate among people with difficult-to-treat HIV was 84% compared with 95% for non-difficult (p < 0.001). This difference was similar both among men and women, and it remained statistically significant after multivariable adjustment (aOR, 0.28; 95% CI 0.22–0.35). People with difficult-to-treat HIV reported worse physical (but not psychological) health, and this also remained statistically significant in multivariable analysis (aOR, 0.74; 95% CI 0.60–0.92).

Conclusion: Although 9% of the HIV cohort in Sweden in 2023 were classified as having difficult-to-treat HIV, a large proportion of these were virally suppressed. Challenges such as advanced resistance and need for salvage therapy are rare in the current Swedish cohort.
Methods: We conducted a cohort study using data from the Aid for AIDS (AFA) private sector disease management program in South Africa and from the NA-ACCORD collaboration of HIV cohorts in the United States and Canada. We included PWH aged 18–84 years with follow-up in AFA (2011–2020) or NA-ACCORD (2000–2020). We computed excess LYL associated with an ICD-9/10 diagnosis for any mental illness, organic disorder, psychotic disorder, bipolar disorder, depressive disorder, and anxiety disorder, by gender and region. Excess LYL measures the average difference in remaining life expectancy in PWH diagnosed with a mental illness compared with PWH of the same age without a mental illness. We disaggregated LYL into natural, unnatural (due to injuries or violence), and unknown causes of death.

Results: We included 126,058 PWH from South Africa (58% women, 4.6 median years of follow-up) and 85,296 from North America (9% women, 5.8 median years of follow-up). In South Africa, 45% of men and 50% of women were diagnosed with a mental illness. In North America, 63% of men and 65% of women were diagnosed with a mental illness. In both regions, depressive and anxiety disorders were the most common. In South Africa, mental illness was associated with 3.5 LYL (95% CI 2.6–4.3) in men and 3.0 LYL (95% CI 1.3–4.6) in women (Figure). In North America, mental illness was associated with 2.9 LYL (95% CI 2.5–3.3) in men and 3.4 LYL (95% CI 1.2–5.9) in women. In men, 68% of the LYL associated with mental illness in North America were attributable to natural causes of death, compared with 79% in South Africa. For women, in both regions the entire excess mortality burden was due to natural causes. The excess LYL ranged from 1.3 (95% CI 0.7–3.2) in South African women with anxiety to 17.2 (95% CI 14.5–19.2) in South African women with an organic disorder.

Conclusion: ICD diagnoses for mental illness were associated with excess mortality in South Africans and North American PWH. Death from natural causes was the main contributor to their excess mortality. These findings support the implementation of strategies for the prevention, early detection, and treatment of mental illnesses in PWH, and for the screening and treatment of physical comorbidities in PWH who have a mental illness.

1049 Modeled Estimates of Disease Burden Attributable to Interactions Between HIV and Depression in Kenya

Daniel T. Citron1, Hae-Young Kim1, Rosco Kasuji1, Samuel Mwaliili1, Josiline Chemutai1, Ingrida Platiai1, Frey Assie1, Anna Bershey2
1New York University Langone Medical Center, New York, NY, USA; 2Makerere University College of Health Sciences, Kampala, Uganda, Strathmore University, Nairobi, Kenya

Background: In sub-Saharan Africa, AIDS is the leading cause of mortality, while depression is the leading cause of morbidity. Depression is known to increase HIV acquisition and impede effective treatment, while people living with HIV have elevated risk of depression, but these interactions have not previously been modeled in the context of overlapping HIV and mental health crises. We used a simulation model to estimate how the HIV pandemic has impacted depression and how the depression epidemic has impacted the HIV pandemic in Nyanza, Kenya, a geography where both conditions are prevalent.

Methods: We adapted a previously validated agent-based network transmission model calibrated to age- and sex-specific prevalence of HIV and coverage of treatment and prevention services. We augmented this model to include major depressive disorder. The model was calibrated to the age- and sex-specific incidence, recovery, and relapse rates of major depressive disorder in Kenya. Calibration was applied to a primary scenario, in which we included the following interactions between HIV and depression based on rapid review of literature: HIV increases incidence of depression (2x); depression increases HIV acquisition rate (1.6x); depression interferes with the HIV care continuum (2x delays to testing, 1.2x reduction in adherence to treatment, and 2x higher rates of treatment discontinuation). In a counterfactual scenario, we removed all interactions between HIV and depression. We estimated the number of episodes of depression, new HIV cases, and HIV-related deaths over 1985–2025.

Results: In the primary scenario, we estimated 1.24 million new HIV infections, 0.66 million HIV deaths, and 17.7 million episodes of depression in western Kenya over 1985–2025. We found a 9.96% (95% CI: 9.84%–10.1%) increase in episodes of depression attributable to HIV; a 5.18% (95% CI: 4.93%–5.44%) increase in new HIV cases attributable to depression; and a 10.5% (95% CI: 10.1%–10.8%) increase in HIV-related deaths attributable to depression. Sensitivity analysis is ongoing to identify interactions most strongly responsible for these effects and that contribute the greatest uncertainties.

Conclusion: Our findings suggest interactions between depression and HIV have substantially exacerbated both epidemics. Research is needed to more precisely quantify strengths of the interactions and how they differ across populations and settings.

1050 Prevalence of Diagnosed and Undiagnosed Depression Among US Adults With HIV

Linda Beer, Linda J. Koenig, Yunfeng Tie, Xin A. Yuan, Jennifer Fagan, Kate Buchacz, John Weiser
Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: People with HIV are disproportionately affected by depression. Effectively diagnosing and treating depression could improve quality of life (QoL) and HIV outcomes. We used data from CDC’s Medical Monitoring Project (MMP) to report nationally representative estimates of diagnosed and undiagnosed depression among U.S. adults with HIV (PWH).

Methods: During 6/2021–5/2022, MMP collected interview data on depression symptoms consistent with a diagnosis using the Patient Health Questionnaire (PHQ-8) and depression diagnoses from medical records of PWH (Figure). We report weighted percentages and prevalence ratios (PRs) with predicted marginal means and 95% confidence intervals (CIs) to quantify differences between groups on key social and health factors.

Results: Overall, 34% of PWH experienced any depression (either by diagnosis or PHQ-8); of these, 26% had symptoms but no diagnosis (undiagnosed depression), 19% had both diagnosis and symptoms, and 55% had a diagnosis without symptoms (Figure). Among those with depression, cisgender men (PR: 1.34, CI: 1.04–1.72) and transgender persons (PR: 1.77, CI: 1.07–2.90) were more likely than cisgender women to have undiagnosed depression, as were those with a disability (PR: 1.52, CI: 1.19–1.94) and food insecurity (PR: 1.67, CI: 1.37–2.03) than those without. Unemployed persons (PR: 1.62, CI: 1.11–2.38) were more likely than employed persons to have diagnosed depression with symptoms, as were those with a disability (PR: 2.78, CI: 1.32–3.64), who experienced housing instability/homelessness (PR: 1.37, CI: 1.06–1.77), food insecurity (PR: 1.46, CI: 1.12–1.90), or discrimination in HIV care (PR: 1.71, CI: 1.30–2.23) than those without. HIV stigma was higher with nonoverlapping CIs among the undiagnosed (median score, range 0–100: 40.4, CI: 34.6–46.2) and diagnosed with symptoms (43.1, CI: 36.2–50.0) than those diagnosed without symptoms (28.0, CI: 26.1–30.0). Those with symptoms (undiagnosed or diagnosed) were less likely than those diagnosed without symptoms to be dose adherent (PR: 0.88, CI: 0.78–0.98, PR: 0.73, CI: 0.60–0.89) or have sustained viral suppression (PR: 0.62, CI: 0.54–0.72; PR: 0.91, CI: 0.82–1.00) and were more likely to have unmet needs for mental health services (PR: 2.38, CI: 1.62–3.51; PR: 2.03, CI: 1.45–2.83).

Conclusion: One-third of PWH experienced depression; nearly half of them were undiagnosed or still experiencing considerable symptoms. Expanding universal screening and high-quality treatment for depression could improve QoL and HIV outcomes.
1051 Trends in Suicide-Related Emergency Department Visits Among People With and Without HIV in Bronx, NY

Chloe Roske,1 Caitlin Hills,1 Wen Zhu B. Mowrey,1 Yingchen Xu,1 Aaron S. Breslow,1 Atul K. Bhattiprolu,1 Ava Eruiler,1 Grishma Patel,1 Joan W. Berman1, Anjali Sharma,2 Vilma Gabbay,2 David B. Hanna1

1Albert Einstein College of Medicine, Bronx, NY, USA, 2University of Miami, Miami, FL, USA

**Background:** People living with HIV (PWH) are at elevated risk for suicidality (i.e., suicidal ideation, plans, attempts), though little is known about population-specific factors driving potential disparities in Emergency Department (ED) visits in the context of suicide. Using longitudinal data from a large Bronx, NY health system, we measured trends and disparities in suicide-related ED visits stratified by gender, age, race/ethnicity and HIV transmission factor.

**Methods:** Using the Einstein-Rockefeller-CUNY CFAR’s Clinical Cohort Database, we identified all ED visits among patients age 17+ years at 4 EDs in the Montefiore Health System between 2016 and 2022, and determined suicide-related visits using ICD-10-CM diagnosis codes for suicidal ideation/behavior. We measured rates of suicide-related ED visits by HIV status. Rates were annualized per 1,000 ED visits, age-standardized to the 2000 US Standard Population and stratified by gender, age, race/ethnicity and HIV transmission factor.

**Results:** Among 1,760,143 unique ED visits (40,475 among PWH) between 2016-2022, 8,994 (506 among PWH) were suicide-related. The overall rate of suicidal ideation/behavior among PWH was 14.9/1,000 ED visits (95% CI 13.3-16.4), compared with S.2/1,000 (95% CI 1.5-1.3) among those without HIV. Rates were consistently higher among PWH versus those without HIV across gender and age categories (Figure). Among PWH, the highest rates were observed among those age 17-39 years (25.6/1,000, 95% CI 22.4-28.9), cisgender men (18.5/1,000, 95% CI 16.4-20.7), vs cisgender women, 6.6/1,000, 95% CI 5.0-8.3), and non-cisgender individuals (30.1/1,000, 95% CI 16.4-45.0). Before the COVID-19 pandemic, both the annual number and rate of suicide-related ED visits had increased steadily over time among PWH, from N=39 (7.8/1,000) in 2016 to N=74 (16.7/1,000) in 2019. Trends showed a further increase as COVID-19 became established, to N=86 (27.4/1,000) in 2020, particularly among PWH age 17-39, before decreasing in 2021 and 2022. Temporal increases in suicidal ideation/behavior were less pronounced among people without HIV.

**Conclusion:** Suicide-related visits were nearly 3-fold higher among PWH compared with those without HIV in this large, urban ED setting, and increased differently over time, even after accounting for temporal changes associated with the COVID-19 pandemic. Younger PWH and transgender individuals may be at particular risk for suicidality; more research into associated factors is needed.

1053 Mind the Gap: Life Expectancy and Mortality in Males & Females With HIV in British Columbia, Canada

Katherine Kooij, Wendy Zhang, Jason Trigg, Nance Cunningham, Michael Budu, Viviane Dias Lima, Kate Salters, Rolando Barrios, Julio Montaner, Robert Hogg

British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada

**Background:** Life expectancy (LE) of people with HIV (PWH) has risen considerably in the last decades, but LE gains among females with HIV have fallen behind.

**Methods:** We examined trends and sex differences in LE and mortality among all PWH in British Columbia (BC), using data from the Comparative Outcomes And Service Utilization Trends study. LE at ages 20, 40, and 55 was calculated using life tables stratified by sex and period (1996-2002, 2002-12, 2012-20). Using multivariable Cox regression, we modelled the association between time in care and mortality in people with AIDS. Mortality was defined as death due to HIV disease, and injuries/external causes (defined using ICD codes).

**Results:** A total of 11,739 males (82%) and 2,534 females (18%) with HIV were included; 92% and 88%, respectively, were ever on ART. LE for males aged 20
increased from 24.5 (95% CI 22.3 – 26.6) in 1996-2002 to 37.1 (35.4 – 38.8) in 2002-12, and to 48.0 (45.7 – 50.3) in 2012-20. LE for females aged 20 increased, but remained lower, from 22.1 (19.9 – 24.4), to 32.8 (30.6 – 34.9), and to 40.9 (37.7 – 44.2). Patterns were similar at age 40 and 55. The sex gap in LE increased over time, both at age 20 and 40 (from 3.2 in 1996-2002 to 5.3 years in 2012-20), but was not as discernible at age 55. In adjusted models, female sex was significantly associated with all-cause mortality, overall and in 2002-12 and 2012-20 (Table). Similarly, in adjusted models, female sex was associated with mortality from non-communicable disease, but not from injuries/external causes and communicable disease. Further adjustment for CD4 count at ART initiation did not affect the associations significantly.

**Conclusion:** In a setting with universal health care and free ART, the sex gap in LE among PWH continues to increase with time. Clinical and socio-economic factors do not explain that this gap in LE and HRs are increasing over time, but they do largely explain differences in mortality from communicable disease and injuries/external causes. If PWI followed the pattern observed among people without HIV in BC, we would expect the gap in LE to favor females. Our work suggests that addressing socio-structural factors may potentially reduce but not reverse the gap.

### Table: Associations between sex, all-cause, and cause-specific mortality.

<table>
<thead>
<tr>
<th>Female sex, adjusted for baseline age and confounders</th>
<th>Male sex, adjusted for baseline age and confounders</th>
<th>Female sex, adjusted for age and confounders</th>
<th>Male sex, adjusted for age and confounders</th>
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<tbody>
<tr>
<td>LE (95% CI)</td>
<td>LE (95% CI)</td>
<td>LE (95% CI)</td>
<td>LE (95% CI)</td>
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<tr>
<td>Overall</td>
<td>Overall</td>
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<tr>
<td>2.34 (1.91 – 2.86)</td>
<td>2.15 (1.96 – 2.35)</td>
<td>2.13 (1.87 – 2.45)</td>
<td>1.99 (1.78 – 2.23)</td>
</tr>
<tr>
<td>Communicable disease mortality*</td>
<td>Non-communicable disease mortality*</td>
<td>Mortality from injuries/external causes*</td>
<td>Mortality from injuries/external causes*</td>
</tr>
<tr>
<td>1.50 (1.19 – 1.91)</td>
<td>1.95 (1.68 – 2.28)</td>
<td>1.55 (1.15 – 2.07)</td>
<td>1.10 (0.82 – 1.48)</td>
</tr>
</tbody>
</table>

*Communicable disease defined by ICD-9 codes 001-099 and ICD-10 codes A00–Z99; non-communicable disease, ICD-9 codes 200-999 and ICD-10 codes A00–Z99; mortality from injuries/external causes, ICD-9 codes 905-996 and ICD-10 codes E960–E989.

### 1055 Age-Adjusted Mortality Rates Among Persons With HIV, by Race and Ethnicity and Cause of Death

Cameron Steainken1, Cassandra O. Schember2, Nannie Song3, Deanna Sykes4, Philip Peters5, Darpun Sachdev6
1California Department of Public Health, Richmond, CA, USA, 2Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** During 2010–2018, the age-adjusted mortality rate (AAMR) among persons with HIV (PWH) in the United States declined by 37%, primarily from a 48% reduction in HIV-associated deaths. We used public health surveillance data to determine how the COVID-19 pandemic affected mortality by race and ethnicity and cause of death (CoD) among PWH in California.

**Methods:** We analyzed death certificate data for PWH from California Vital Records during 2018–2021. We categorized immediate COD using International Classification of Diseases, Tenth Revision codes and excluded records coded as AAMR. 100,000 persons were calculated by race and ethnicity and by CoD. We calculated percentage changes in AAMRs from 2018–2019 (before COVID-19 pandemic) to 2020–2021 (during COVID-19 pandemic) by race and ethnicity and CoD.

**Results:** AAMR among PWH in California increased 14.6% from 2018–2019 (4.1 deaths/100,000 persons) to 2020–2021 (4.7 deaths/100,000 persons), with larger increases among multiracial (31.2%), Latinx (29.2%), Asian (16.3%), and Black (15.3%) PWH, compared with White (5.2%) PWH (Figure 1). Leading CoD among PWH during both 2018–2019 (29.5% of deaths) and 2020–2021 (25.4% of deaths) periods was HIV. AAMR because of HIV decreased 2.4% from 2018–2019 to 2020–2021. HIV-associated AAMR decreased among White (7.9%) and Latinx (-0.2%) PWH, but increased among multiracial (13.9%), Asian (9.0%), or Black (4.8%) PWH. Overdose rose from the third-leading CoD (6.3% during 2018–2019) to second-leading CoD (8.8% during 2020–2021), with overdose AAMR increasing by 63.7%. Black (84.1%), multiracial (83.1%), and Latinx (77.4%) PWH had higher overdose AAMR increases, compared with White (41.7%) PWH. COVID-19 was the fourth-leading CoD (5.9%) among PWH during 2020–2021. COVID-19 AAMRs were higher among Black (0.7/100,000 persons), multiracial (0.7/100,000 persons), and Latinx (0.5/100,000 persons) PWH, compared with White PWH (0.1 deaths/100,000 persons).

**Conclusion:** Mortality increased among PWH in California, particularly among non-White populations. Overall, HIV mortality declined, but increased among certain races and ethnicities. Although COVID-19 contributed to the pandemic mortality increase, overdose death rates increased substantially across all races and ethnicities, compared with prepandemic years. Interventions directed at overdose-related deaths and disparities in HIV mortality, primarily for Black and multiracial PWH, might help reverse these pandemic-era mortality trends in California.
1057 Registered Causes of Death Remain Largely Unknown Among People With HIV in Latin America

Yanink Cara-Vega1, Antonio Pacheco1, Karu Jayathilake1, Gabriela Carriquiry1, Paula M. Luz1, Diana Machado1, Jorge Pinto1, Claudia P. Cortes1, Carina Cesar1, Marco T. Luque2, Vanessa Rouzier11, Stephany Duda3, Peter J. Rebeiro4, for the Caribbean, Central, and South American Network for HIV Epidemiology (CASCANet)

1Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico; 2Cayo Cruz Foundation — Ilocruz, Bay de Jena, Brazil; 3Vanderbilt University, Nashville, TN, USA; 4Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru; 5Universidade Federal de São Paulo, São Paulo, Brazil; 6Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; 7Fundación Arranque, Santiago, Chile; 8Fundación Hasieng, Buenos Aires, Argentina, 9Instituto Hondureño de Seguridad Social, Tegucigalpa, Honduras, 10G4S8XK0, Port-au-Prince, Haiti

Background: Describing cause-specific mortality helps define public health prevention priorities. However, vital status records may omit causes of death among people with HIV (PWH), even HIV itself or associated conditions, particularly in resource-limited settings. We therefore described mortality among PWH in the Caribbean, Central and South America network for HIV epidemiology (CASCANet) and identified characteristics associated with unknown causes of death.

Methods: Mortality and cause-of-death data from adult PWH in care in CASCANet from 2003-2022 were extracted from local vital status registries and electronic health records (by ICD-10 code). Causes of death, whether primary, secondary, underlying, or contributing, were categorized as: 1. missing/unknown, 2. HIV or AIDS-defining events (ADEs; if ≥1 cause was: pneumonia, TB, other opportunistic infection, ADE malignancy, unspecified HIV/AIDS), or 3. non-ADEs (NADEs; all other causes, including: renal, liver, cardiovascular, NADE malignancy, trauma, etc.). We described overall mortality and compared characteristics by cause-of-death groups using 2 and Kruskal-Wallis tests.

Results: Among 56,620 PWH, there were 6,507 (11%) deaths: 3,283 (50%) unspecified (including 39% entirely missing), 2405 (37%) ADE, and 819 (12%) NADE causes. ADE causes included: 1403 (58%) unspecified HIV/AIDS, 477 (20%) unspecified pneumonia, 276 (11%) TB, 212 (9%) other opportunistic infections, and 125 (5%) ADE malignancies. Common NADE causes were: 284 (35%) NADE infectious diseases, 123 (15%) NADE malignancies, and 111 (13%) traumas. In the unknown-cause group, 45% were women vs. 29% in ADE and 26% in NADE groups. Median age at death was 42 (IQR:33-52) years in unknown-cause vs. 41 (IQR:33-50) in ADE and 47 (IQR:38-56) in NADE groups; median CD4 count at death was 84 (IQR:25-236) cells/μl in unknown-cause vs. 64 (IQR:18-185) in ADE and 211 (IQR:65-421) in NADE groups. Percentage on ART at death was highest in the unknown-cause group (91% vs. 72% for ADE and 83% for NADE groups). P-values were <0.01 for all comparisons.

Conclusion: Of the 11% of the cohort that died over two decades, more than half were recorded as from unknown causes, including a third completely missing, and 58% of ADE causes had an unspecified HIV/AIDS code. Unknown causes occurred mostly in women, younger PWH, and those with lower CD4 counts, similar to those dying from an ADE. More complete cause-of-death data is needed to better identify factors associated with, and prevent deaths due to, ADEs.

1056 Mortality Among People Living With HIV in India: Emergence of Noncommunicable Diseases

Manish Bamrotiya1, Neha Garg2, Alice Marak2, Jade Bell2, Maria Salvat Ballester3, Allison M. McFall2, Shruti H. Mehta4, Nidhi Kesarwani4, Bhawani Kushwaha4, Chinnmoyee Das4, Pushpa Kumari3, Hekali Zhimomi2, Manish Bamrotiya1

1The Johns Hopkins University School of Medicine, Baltimore, MD, USA; 2IYR Gattande Center for AIDS Research and Education, Chennai, India; 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 4National AIDS Control Organisation, New Delhi, India

Background: Expanded access to antiretroviral therapy (ART) has significantly reduced HIV-related mortality in adults and children worldwide including a 77% decline in India between 2010 to 2021. Even with this reduction, from January 2019 to March 2020, 79,755 deaths among PLHIV were reported across all government ART centers in the country. While mortality patterns among PLHIV show a shift toward noncommunicable diseases (NCDs) in high income settings, limited data is available in LMICs. We describe the emergence of NCDs as a cause of death among PLHIV in India.

Methods: Between January 2019 and March 2020, we collaborated with India’s National AIDS Control Program to perform verbal autopsy assessments, a method to determine the most probable cause of death by interviewing caregivers of deceased PLHIV when official medical certification is not available. Interviewers employed the WHO-VA-2016 tool, and two independent physicians utilized ICD classifications to determine the cause of death.

Results: Among the 1,001 deaths investigated, the median age was 42 years (IQR: 35-50), 68% were men and most deaths occurred in Northern India (32%). The primary cause of death was HIV-related opportunistic infections (76%) with tuberculosis counting for 31% of those, followed by cancer (7%), heart disease (6%), renal disease (2%), and liver disease (1%). However, for those who on ART for longer durations, the relative contribution of NCDs was higher — NCDs accounted for only 8% of mortality among those on ART for <1 year compared to 33% among those on ART for more than 5 years (p<0.001). People who died of NCDs were significantly older compared to those who died from OIs (p<0.05). The most common form of malignancy in men was head and neck cancer (24%), while cervical cancer (27%) was the most common in women.

Conclusion: Mortality attributable to AIDS-related OIs, particularly TB, remains high in this setting, particularly among individuals who are newly initiating ART. This reflects a continued need to detect and engage PLHIV early after infection and support high levels of adherence. As people living with HIV age, it is likely that the relative contribution of NCDs, including cancer, continues to increase highlighting the importance of screening and managing these conditions within ART programs in LMICs.

1058 Predicting Cause-Specific Mortality With the VACS Index 2.0 Among Persons With HIV

Julie Ambier1, Suzanne M. Ingel1, John Gill2, Sophie Abgrall3, Mojgan Hassamfar1, Peter Reiss1, Christopher Wyers4, Heidi M. Crane5, Inma Jarrin6, Michael J. Silverberg7, Kathleen A. McGuinness8, Amy C. Justitie9, Jonathan A. Sterne1, Adam Trickey1, for the Antiretroviral Therapy Cohort Collaboration (ART-CC)

1University of Bristol, Bristol, United Kingdom; 2University of Calgary, Calgary, Canada; 3University of Paris-Sud, Orsay, France; 4University of Bordeaux, Bordeaux, France; 5University of Amsterdam, Amsterdam, Netherlands; 6University of Cologne, Cologne, Germany; 7University of Washington, Seattle, WA, USA; 8Institute of Health Carlos III, Madrid, Spain; 9Kaiser Permanente Northern California, Oakland, CA, USA

Background: Predicting cause-specific mortality among persons with HIV (PWH) could facilitate targeted care to improve survival. We assessed discrimination of the Veterans Aging Cohort Study (VACS) Index 2.0 in predicting cause-specific mortality among PWH on antiretroviral therapy (ART) in Europe and North America.

Methods: The VACS Index 2.0 consists of thirteen variables: age, sex at birth, body mass index (BMI), CD4 count, creatinine, HIV-1 RNA viral load, haemoglobin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelet count, white blood cell count, albumin, and hepatitis C infection. Using data from 12 cohorts contributing to the Antiretroviral Therapy Cohort Collaboration, VACS 2.0 was calculated for PWH who initiated ART between
2000 and 2018, around a randomly selected visit date within the period 1 year after ART initiation to last visit. Missings in VACS 2.0 variables was addressed through multiple imputation. We estimated associations between VACS 2.0 and specific causes of death using Cox models, with discrimination evaluated using Harrell’s C-statistic. Absolute mortality risks were modelled with flexible parametric survival models.

Results: Among 59,741 PWH, 80% were men and at follow-up start mean age was 43 and mean VACS 2.0 score was 41.0. VACS 2.0 values were measured median of 3.2 years after ART initiation and ranged from 0 to 128; higher values indicate worse prognosis. There were 3,117 deaths during 217,257 person-years (median follow-up was 2.6 years). Non-AIDS-defining cancers were the most common cause of death (n=569), followed by AIDS (n=527). Discrimination for five-year all-cause mortality was C=0.83. Discrimination for specific causes of death was highest for deaths due to AIDS (0.91), liver-related causes (0.90) and respiratory causes (0.88). Discrimination was lowest for suicides/accidental deaths (0.65), unclassifiable causes (0.76), cardiovascular deaths (0.77) and “other” causes (0.78). Predicted probabilities of 5-year mortality were low for except for PWH with very high (>90) VACS 2.0 values e.g. the 5-year probability of AIDS death for those with VACS 2.0 score 40 was 0.1%, increasing to 7.6% for scores of 90.

Conclusion: To improve discrimination for causes for which discrimination is lower, future VACS Index analyses could incorporate additional measures, such as psychopathological risk factors, extra biomarkers (for example, lipid profiles and inflammatory markers), or conditions included in the Charlson Comorbidity Index.

1059 Care Interruptions and Mortality Among Adults on Antiretroviral Therapy in Europe and North America
Adam Trickey1, Christopher T. Rentsch2, Nikos Pantazis3, Rebeca Izquierdo4, Andrea Antonini5, Gesela Leierer6, Greer Burkholder7, John Gill8, Marc Van der Vaal9, F. Bonnet10, Heidi M. Crane11, Michael J. Silverberg12, Suzanne M. In-gle13, Jonathan A. Sterne14, for the Antiretroviral Therapy Cohort Collaboration (ART-CC).1 University of Bristol, Bristol, United Kingdom, 2 Yale University, New Haven, CT, USA, 3 University of Athens, Athens, Greece, 4 Institute of Salud Carlos III, Madrid, Spain, 5 IRCSS Lazzaro Spallanzani, Rome, Italy, 6 Medical University of Innsbruck, Innsbruck, Austria, 7 University of Alabama at Birmingham, Birmingham, AL, USA, 8 University of Calgary, Calgary, Canada, 9 Stichting HIV Monitoring, Amsterdam, Netherlands, 10 University of Bordeaux, Bordeaux, France, 11 University of Washington, Seattle, WA, USA, 12 Kaiser Permanente, Oakland, CA, USA

Background: Interruptions to the care of people with HIV (PWH) on antiretroviral therapy (ART) are associated with adverse outcomes. Studies to date mostly relied on composite outcomes, such as AIDS and death, due to insufficient number of iindi-vidual outcome events. We investigated whether mortality rates following a care interruption differed from mortality rates after first starting ART.

Methods: Data from 2004-2020 were combined from 18 European and North American HIV cohort studies of adult PWH starting ART between 2004-19. We defined in-hospital interruptions to HIV specialized health care as breaks of ≥365 days duration (no lab, CD4, RNA, ART, or visit records), with a subsequent return to care without a suppressed viral load; distinct from loss-to-follow-up. In sensitivity analyses, we used breaks of ≥180, ≥270, and ≥535 days. Follow-up time for each PWH was allocated across three groups: “no previous interruption”, and “early” or “late in-interruption” (reinitiating after a gap starting <6 or ≥6 months of first ART initiation). Each PWH contributed follow-up to the pre/no interruption group. We used Cox regression to compare mortality rates across the three groups, adjusting for sex, HIV acquisition mode, year of ART initiation/re-initiation, and time-updated age and CD4 count on initiation or re-initiation of ART.

Results: Among 89,197 included PWH, median age at ART start was 39 years (IQR 31-48) and 83% were male. 7796 (9%) of PWH had at least one care interruption. There were 1300 “early interruption” group episodes (1300 PWH), and 9773 “late interruption” group episodes (7832 PWH). Median CD4 count at ART start was 280 (IQR: 143-417), while after early and late interruptions (where available) it was 235 (IQR 95-456) and 352 (IQR 150-600) cells/μl, respectively. There were 6098 deaths in 536,870 person-years (rate 11.4 (95% CI 11.1-11.7) per 1000 person-years). Adjusted hazard ratios for the early and late interruption groups were 1.69 (95% CI: 1.39-2.06) and 1.80 (95% CI: 1.63-1.98), respectively, compared with the no/pre-interruption group. Results were robust in sensitivity analyses (≥180, ≥270 and ≥535 days).

Conclusion: Mortality was higher among PWH reinitiating ART following an interruption with unsuppressed viral loads, compared with when PWH initially start ART, indicating the importance of full adherence to ART. The mechanism for this higher mortality requires further investigation as care interruptions may be associated with other factors linked to higher mortality.

1060 Impact of Accessing Care at an Advanced Stage on Mortality in PWH in France, 2002-2016
Valérie Potard1, Malamine Gassama2, Emilie Lanoy3, Sylvie Abel4, Firouze Bani Sadr5, Sylvie Bregenzer6, Fabienne Caby7, Blandine Denis8, Pierre de Truchis9, Guillaume Martin-Blandel10, Lionel Piron11, Axel Versuchen11, Dominique Costagliola12, Sophie Grabar13, for the ANRS C04 FHDH Late Presentation Study Group
1 Sorbonne Université, Paris, France, 2 Centre Hospitalier Universitaire de Fort de France, Fort de France, Martinique, 3 Centre Hospitalier Universitaire de Reims, Reims, France, 4 Ass-Université de Rennes, Rennes, France, 5 Centre Hospitalier Universitaire de Dijon Bourgogne, Dijon, France, 6 Centre Hospitalier Universitaire de Strasbourg, Strasbourg, France

Background: Previous studies have shown the deleterious impact of access to care with an advanced HIV-disease (CD4 ≤200) or AIDS, no primary infection on the mortality risk in people living with HIV (PWH). Here, we explored the respective impact of access to care with AIDS or with CD4 ≤50/mm³ without AIDS or with CD4 50-200/mm³ without AIDS on the mortality risk up to 5 years after the first access to care, and whether availability of new antiretroviral regimens led to a smaller impact.

Methods: Adult participants newly included in the ANRS-C04-FHDH cohort between 2002-2016, with HIV-1 infection were selected. Besides the 3 categories of advanced HIV-disease at access to care, 2 others were defined as follows: intermediate HIV-disease as CD4 between 200-350/mm³ without AIDS and early HIV-disease as either CD4>350/mm³ without AIDS, or primary infection. The impact of the stage at first access to care on the mortality risk was analyzed by using Fine & Gray competing risk models considering lost to follow-up ≥18 months as a competing event. Follow-up after access to care was categorized into 0 – 6, 6 – 12, 12 – 24, 24 – 48, 48 – 60 months. Models were adjusted for age, sex, acquisition mode, region of origin, delay between diagnosis and access to care and period of access to care (2002-2013 vs 2014-2016).

Results: Among the 64400 PWH included, 18305 (28.4%) presented with an advanced HIV-disease and 13042 (20.3%) with an intermediate HIV-disease. At 60 months, the cumulative incidence of death was estimated as 1.8% (95% CI: 1.7–1.9) overall, from 6.0% (95% CI: 5.4–6.7) among those with AIDS to 0.9% (95% CI: 0.8–1.0) among those with early HIV-disease (Table). Compared to people with an early HIV-disease, those with AIDS had a very high risk of death, with a sub-distribution hazard ratio (SHR) of 18.4 (95% CI: 12.0-28.4) in the first 6 months of follow-up, which remained significant 48-60 months after inclusion 2.1 (95% CI: 1.3-3.3) (Table). In the other categories of advanced HIV-disease, the risk of death was also significantly higher while to a smaller extent. There was no statistical difference between calendar periods.

Conclusion: A delayed access to care remains associated with an increased risk of death even after 48 months of follow-up. There was no significant improvement in the risk of death after introduction of integrase inhibitors for combined antiretroviral initiation in 2014.
1061 Trends in Hospital Readmission Among People With and Without HIV in the US, 2010-2020

Xianming Zhu1, Eshan U. Patel2, Mary Kate Grabowski3, Thomas C. Quinn4, Stephen A. Berry1, Kelly A. Geba1, Aaron A. R. Toban1
1The Johns Hopkins University, Baltimore, MD, USA; 2The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 3National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA

Background: Thirty-day readmission is a prominent US hospital quality metric. However, there are limited data to compare readmission trends among people with HIV (PWH) to people without HIV (PWoH) in the era of universal antiretroviral therapy in the US.

Methods: We used data from the 2010 to 2020 Nationwide Readmission Database (NRD), the largest readmission database in the US that includes ~18 million unweighted hospitalizations each year and represents ~60% of all US hospitalizations. Following Centers for Medicare & Medicaid Services methodology, we excluded those age<18 years; discharged dead, against medical advice or without 30-day post-discharge follow up window, and admissions for primary psychiatric diagnoses, rehabilitation, cancer treatment or COVID-19. The outcome was the probability of 30-day all-cause unplanned readmission since discharge from a prior (index) admission. A readmission could also be an index admission. ICD-9/ICD-10 codes were used to identify PWH. Crude readmission probability was estimated for PWH and PWoH each year. Subgroup analyses were stratified by age, sex, and median ZIP code household income. Survey weights were applied to all analyses to generate nationally representative estimates.

Results: The study population included 25,205,538 (weighted) index admissions in 2010, 24,338,782 in 2019, and 21,258,399 in 2020. In 2010 and 2020, PWH contributed 140,014 (0.56%) and 126,029 (0.59%) index admissions, respectively. Overall, PWH had higher readmission risk than PWoH. The readmission probability for PWH decreased gradually from 23.9% in 2010 to 20.3% in 2020. For PWoH, the readmission probability was stable except during 2015, the year of transition from ICD-9 to ICD-10. Stratified by sex, female PWH had slightly higher readmission probability than male PWH and the difference fluctuated over time. However, female PWoH continued to have similar lower readmission probability than male PWoH (Figure). Older PWoH consistently had higher readmission risk than younger PWoH. In contrast, different age groups of PWH had similar readmission risk over time. PWoH residing in areas with the lowest median household incomes had the highest readmission risk for all the years.

Conclusion: The quality of hospital care for adult PWH in the US has improved in the past decade, but there is still a significant gap in readmission risk between PWH and PWoH, especially among women.

1062 Wastewater Monitoring of HIV-1: Feasibility and Comparison to Surveillance Data

Marlene K. Wolfe1, Meri Varkila1, Julie Parsonnet3, Alexandra B. Boehm1
1Emory University, Atlanta, GA, USA; 2Stanford University, Stanford, CA, USA

Background: Wastewater-based epidemiology (WBE) is being used to identify and quantify infectious agents circulating in communities without the need to test individuals. HIV has previously been detected in wastewater and HIV RNA and DNA have both been amplified from urine and feces of people living with HIV (PLWH). Thus, measuring HIV in wastewater appears feasible, but has not been used for the purpose of monitoring HIV in communities.

Methods: We applied a previously developed hydrolysate-probe based PCR assay targeting the LTR region of HIV-1 to quantify nucleic acids (NA) in wastewater settled solids using droplet digital (RT-)PCR. We performed retrospective monitoring of HIV-1 concentration in longitudinal wastewater samples from two publicly owned wastewater treatment plants, one in San Francisco (OSP) and the other in San Jose (SJ) between February 2021 and April 2023. Samples were collected every week. To assess concordance between wastewater data and local surveillance data from public health departments, we compared trends in wastewater HIV-1 concentrations to HIV prevalence estimates per county.

Results: Highly abundant HIV-1 NA were detected in 94% (215/230) and 23% (53/229) of samples in OSP and SJ sewersheds, respectively. Samples from the OSP sewered consistently yielded higher concentrations of HIV NA than samples from SJ (OSP median 7.3*10^6 cp/g vs. SJ median non-detect). Samples from SJ and OSP delivered non-detect to 1.1*10^6 cp/g, figure 1 mirroring surveillance estimates of higher community prevalence of HIV in San Francisco County than in Santa Clara County in 2021 (1334.1 and 190.6 PLWH per 100,000 population, respectively). We observed similar concentrations of HIV-1 NA in wastewater samples with and without a reverse transcription step during PCR suggesting that most, if not all, of measured NA in wastewater is HIV-1 DNA rather than RNA.

Conclusion: Our findings demonstrate the feasibility of monitoring HIV concentrations in communal wastewater and show good concordance with local surveillance data on HIV prevalence. Results from wastewater can be used to obtain information on HIV at a localized, community level and could serve as a complementary approach to existing HIV surveillance frameworks helping identify priority areas for intervention. Further work by our group will investigate dynamics of viral shedding in PLWH, develop assays for measuring antiretroviral drug-resistance in wastewater, and identify the optimal uses of wastewater surveillance of HIV-1.

Figure: Thirty-day all-cause unplanned hospital readmission probability among people with and without HIV by sex in the US, 2010-2020.

1063 Statewide Real-Time Integration of Molecular and Contact Tracking Data to Disrupt HIV Transmission

Rami Kantor1, Jon Steingrimsson1, John Fulton1, Vlad Novitsky2, Mark Howison3, Fizza Gillani1, Lila Bhattachari1, Meghan MacAskill1, Joel Hague1, August Guang1, Aditya Khanna1, Casey Dunn1, Joseph Hogan1, Thomas Bertrand4, Utpala Bandy1
1Brown University, Providence, RI, USA; 2Research Improving People’s Lives, Providence, RI, USA; 3Yale University, New Haven, CT, USA; 4Rhode Island Department of Health, Providence, RI, USA

Background: Tools beyond contact tracing are still needed to disrupt HIV transmission. Molecular cluster analysis helps stop outbreaks, but its precise benefit to routine public health actions is an existing knowledge gap. We hypothesized that integration of statewide molecular data with contact tracing by routinely re-interviewing new diagnoses who cluster molecularly will increase motivation and enhance contact tracing.

Methods: To address the hypothesis we (1) built an academic-governmental partnership in Rhode Island (RI); (2) maximized statewide representation of HIV-1 pol sequences; (3) developed an automated bioinformatics pipeline to aggregate de-identified data across systems; and (4) used phylogenetic tools to infer clusters in near-real-time, identify all new diagnoses who cluster, routinely attempt to re-interview and inform them of clustering, and assess this intervention’s impact (1st interviews of all RI new diagnoses occur before sequence availability).

Results: In a 2-year (Jan ‘21-Dec ’22) study, of 100 new RI diagnoses, 52 were in molecular clusters. Re-interviews were feasible for only 22/52 (42%), revealing an important gap. Of the 22, only one provided new data, rejecting...
1064 Incident HIV Infection Drives Community HIV Cluster Growth
Antoine Chailliot1, Alan Wells1, Tom Chen1, Ravi Gayal1, Samantha Tweeten1, Sanjay R. Mehta1, Susan J. Little1
1University of California San Diego, San Diego, CA, USA, 2Harvard Pilgrim Health Care Institute, Boston, MA, USA, 3Public Health Services - County of San Diego, San Diego, CA, USA

Background: Molecular HIV surveillance (MHS) is routinely used by U.S. public health departments to monitor HIV transmission dynamics within populations and regions. We analyzed MHS data to identify potential drivers of transmission by investigating genetically related infections (transmission clusters).

Methods: De-identified data were obtained from the epidemiology unit of the Health and Human Services Agency (HHSAs) of San Diego County. A baseline HIV genetic network was inferred based on genetic distance of 0.5% (proxy for recent linkage) for all HIV diagnoses from 2006-2016, and links from newly diagnosed people with HIV (PWH) were added to the network from 2017-2023. The presence of a negative HIV test within 6 months of a new HIV diagnosis was used to indicate incident HIV infection. Cox proportional hazards models were used to identify factors associated with the rate of transmission cluster growth. These results were used to predict the possible impact of hypothetical interventions focused on attributes of clustering PWH.

Results: Among 3,676 individuals with diagnosed HIV, 1,938 PWH had at least one HIV sequence, collected a mean of 109 days following the date of HIV diagnosis (IQR 36). Overall, 16% of sequences were linked in 115 clusters (median cluster size 2; range 2-9). Cluster growth was strongly associated with larger size of the cluster (HR 2.5; 95% CI 2.1, 2.8), proportion of PWH in the cluster with incident infection (HR 3.0; 95% CI 1.6, 5.4), and proportion with a bacterial sexually transmitted infection (STI, including gonorrhea and syphilis) within 1 year of HIV diagnosis (HR 1.7; 95% CI 1.1, 2.6) in univariate analysis. Only larger cluster size (HR 2.4; 95% CI 1.4, 4.4) were still associated with cluster growth in multivariable analysis (Table). Proportion of PWH with unsuppressed viremia (VL>200 copies/ml) in a cluster was not associated with growth of that cluster. None of the theoretical interventions evaluated were shown to significantly reduce the predicted rate of cluster growth over the subsequent three years.

Conclusion: This is the first use of public health data demonstrating that incident infection is a driver of community HIV cluster growth. No special testing for incident infection was required, only the reliance on previous negative HIV test results. In contrast to published reports, these data suggest that incident infection may drive ongoing community HIV transmissions.

1065 Contribution of HIV Transmission Bursts to Future HIV Infections, United States
Rachael Billock1, Anne Marie Francé1, Neeraja Sadavula1, Nivedha Pannier1, Alexandra M. Øster1, Camden J. Hallmarked, Joel O. Wertheim1
1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Seton Health Group, Inc, Atlanta, GA, USA, 3University of California San Diego, La Jolla, CA, USA

Background: HIV clusters show heightened transmission rates and may contribute disproportionally to new infections. However, the influence of these periods of rapid transmission on future HIV infections and the populations that they affect are incompletely characterized.

Methods: Using subtype B HIV pol sequences from the U.S. National HIV Surveillance System reported through 2022 for people with HIV (PWH) diagnosed during 2014–2019, we inferred separate, time-scaled phylogenetic trees with ETE3, FastTree2, and TreeTime for six geographic regions composed of 14 jurisdictions with >50% sequence completeness. We detected transmission bursts, defined as ≥3 connected internal nodes (or transmission events) in a 2-year detection period. We first calculated the relative contribution of transmission bursts in a fixed detection period to future transmission in a follow-up period by dividing the number of internal nodes during 2017-2019 descended from bursts by the number of lineages associated with bursts during 2015-2016, compared to non-burst-descended internal nodes divided by non-burst-associated lineages. To characterize PWH diagnosed in the follow-up period who were ever members or descendants of a transmission burst, we then detected bursts within any sliding 2-year period during 2014–2019 and identified populations overrepresented among PWH associated with transmission bursts.

Results: The 2,795/86,006 (3.2%) lineages (or persons) associated with a transmission burst during 2015–2016 contributed to 493/3,926 (12.6%) transmissions during 2017–2019 across all jurisdictions. Lineages associated with transmission bursts were 4.3 times as likely as lineages not associated with bursts to contribute to future transmissions. Among PWH diagnosed during 2017–2019, 5,603/43,721 (12.8%) were ever members or descendants of transmission bursts during 2014–2019. Groups overrepresented among members or descendants of transmission bursts (i.e., >12.8%) included PWH aged 13–19 (24.5%) or 20–29 years (16.6%) at HIV diagnosis, diagnosed during acute or early HIV infection (17.9%), who reported male-to-male sexual contact (15.3%), or who were transgender, non-binary, or another gender (15.3%).

Conclusion: Lineages associated with transmission bursts contribute disproportionately to future transmission, underscoring the value of detecting and responding to clusters to prevent transmissions. Bursts of rapid HIV transmission likely contribute to disparities in overall HIV incidence for some key populations.
impacts of PrEP on HIV transmission are not fully understood. We hypothesized that widespread PrEP availability in British Columbia (BC), Canada since 2018 has averted hundreds of new HIV cases heterogeneously distributed across phylogenetic clusters.

**Methods:** Using data from the BC Drug Treatment Program, we aligned 42043 HIV partial pol sequences from 10740 individuals to the HXB2 reference and removed surveillance drug resistance mutations. Phylogenetic trees were inferred to identify clusters with 5 or more members with pairwise tree distance less than 0.02 substitutions/site. New diagnoses within clusters over time were used to estimate cluster-specific reproduction numbers (Re) over time. We tested whether PrEP availability significantly affected Re after 2018 in generalized estimating equations, adjusted for cluster size, median age, risk group composition, COVID-19, and treatment guidelines. We summarized characteristics of clusters without reduced growth following PrEP as missed opportunities. For clusters with significantly reduced Re, we fit counterfactual models to data preceding PrEP to predict growth in the absence of PrEP, with consideration for COVID-19. Predicted cases without PrEP were compared to observed cases to quantify cases averted via PrEP.

**Results:** BC HIV phylogenetic clusters exhibited differential growth and Re since widespread PrEP availability in 2018. Re of most clusters comprised predominantly of men who have sex with men (MSM) have declined since 2018. However, we identified missed opportunities for PrEP among a large cluster of young MSM with Re above 1 between 2016 and 2020, and in a cluster comprised primarily of people who use drugs (PWUD) with elevated Re from 2020-2022. Preliminary models of cluster growth in the absence of PrEP suggest averted HIV cases were heterogeneously distributed across phylogenetic clusters.

**Conclusion:** These results highlight the success of the PrEP program in averting new HIV cases, while emphasizing clusters and sociodemographic groups that could benefit from prioritized access and assistance with adherence to PrEP.

### Geospatial and Phylogenetic Clustering of Acute HIV Infections in Lilongwe, Malawi

**Griffin J. Bell, Kimberly Powers, Oliver Ratmann, Ann M. Dennis, Pearson Mmudzi, Mitch Matoga, Edward Jere, David Bonsall, Sharon Weir, Michael Emch, Irving F. Hoffman, Myron S. Cohen, William Miller**


**Background:** HIV transmission spikes during the first months of infection, especially during acute (pre-seroconversion) HIV infection (AHI). Rapid propagation of HIV during early infection hinders universal test and treat interventions, which typically identify infections after the period of elevated transmission risk. To guide prospective interventions against transmission during this period, we evaluated the geospatial and phylogenetic clustering of acute and early infection in Lilongwe, Malawi.

**Methods:** We identified 144 persons with AHI, 30 people who recently acquired HIV (determined with the Sedia LAg-Avidity EIA), and 652 people with chronic HIV who came to a Lilongwe STI clinic between 2015 and 2019. We mapped the point locations of households with an AHI case and 721 sex-exchange venues identified with the PLACE method. To evaluate the spatial clustering of AHI, we used Tango and Takahashi’s flexible scan statistic (α=0.2). Consensus HIV sequences were obtained from blood samples with the veseq-HIV protocol and shivered and aligned with MAFFT. Maximum-likelihood trees were built with IQ-TREE. Monophyletic sequences with genetic distances <5.3% were considered phylogenetic clusters.

**Results:** We identified 6 spatial areas (0.2-1.7 km²) in Lilongwe where household locations of people with AHI were overrepresented, comprising 38% of AHI cases in 1% of the populated land area. These spatial areas contained 57 reported sex-exchange venues and were highly connected: the contiguous M1 and S124 roads run directly through 4 spatial clusters. Although viral sequencing failed for 39% of people with AHI and 60% with recent or chronic infections, we still identified 13 nonoverlapping two-person clusters in our phylogenetic analysis. Four pairs (3 acute-acute, 1 acute-recent) attended the clinic 0-95 days apart, suggesting transmission during acute and early infection. A fifth recent-recent pair (270 days apart) also suggested early transmission. Acute-acute pairs lived 0.2-4.5 km apart, but not in the same spatial clusters.

**Conclusion:** Spatial clustering of AHI exists in highly connected areas with sex-exchange venues in Lilongwe, suggesting that spatially focused interventions could be key in pursuit of HIV elimination. Evidence of extensive AHI transmission chains was limited: no phylogenetic cluster had >2 members. Mirroring earlier modeling estimates of the role of AHI in Lilongwe, 5 of 13 (38%) phylogenetically linked pairs suggested transmission during acute and early HIV infection.

### Increasing Intra- and Inter-Subtype HIV Diversity Despite Rapidly Declining HIV Incidence in Uganda

**Seungwon Kim, Michael A. Martinin, Ronald M. Galwango, Olivier Leyeendecker, Thomas C. Quinn, Godfrey Kigozi, Robert Ssekubuya, David Bonsall, Steven J. Reynolds, Brian Foley, Lucie Abeler-Dörner, Christophe Fraser, Olivier Ratmann, Joseph Kagaayi, Mary Kate Grabowski**

*The Johns Hopkins University School of Medicine, Baltimore, MD, USA, *Aliku Health Sciences Program, Rakai, Uganda, *National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA, *University of Oxford, Oxford, United Kingdom, *Los Alamos National Laboratory, Los Alamos, NM, USA, *Imperial College London, London, United Kingdom, *The Johns Hopkins University, Baltimore, MD, USA*

**Background:** HIV incidence has been declining in sub-Saharan Africa with scale-up of HIV interventions. However, there is limited data on HIV evolutionary trends in African populations with declining epemics. Here, we evaluated changes in HIV diversity over a twenty-five-year period spanning the introduction and scale-up of HIV prevention and treatment programs in Uganda.

**Methods:** We used HIV sequence and survey data from the Rakai Community Cohort Study, a longitudinal population HIV surveillance cohort in southern Uganda. p24 and gp41 consensus sequence data were generated from blood samples of persons living with HIV (PLHIV) in 31 inland semi-urban trading and agrarian communities (1994 to 2018) and four hyperendemic Lake Victoria fishing communities (2011 to 2018) under continuous surveillance. HIV subtype was assigned using the Recombination Identification Program with phylogenetic confirmation. Inter-subtype diversity was estimated using the Shannon diversity index and intra-subtype diversity with the nucleotide diversity and pairwise TN93 genetic distances. Evolutionary dynamics were assessed among demographic and behavioral sub-groups and by migration status.

**Results:** HIV genomic data were available from 4,999 PLHIV, including 3,060 and 1,939 persons residing in inland and fishing communities, respectively, and from 1,484 HIV seroincident cases. In inland communities, subtype A1 viruses proportionately increased in p24 from 17% in 1995 to 32% in 2018 (p<0.001) and in gp41 from 21% in 1995 to 48% in 2018 (p<0.001), while those of subtype D declined in p24 from 80% in 1995 to 45% in 2018 (p<0.001) and in gp41 from 76% in 1995 to 38% in 2018 (p<0.001). In both genes, an increasing proportion of viruses were classified as recombinants (e.g., in p24 from 2.7% in 1994 to 21.4% in 2018 in inland communities). While p24 intra-subtype genetic diversity leveled off after 2014, diversity of gp41 increased through 2018. Inter- and intra-subtype viral diversity generally increased across all population sub-groups, including among individuals with no recent migration history or extra-community sexual partners.

**Conclusion:** Although HIV incidence has declined in Uganda, intra and inter-subtype HIV diversity has increased. Continued molecular surveillance may provide a better understanding of the dynamics driving population HIV evolution and yield important insights for epidemic control.
1069 HIV-1 A6 Variant Transmissions Poland Are Fuelled by War Refugees From Ukraine & Local MSM Clusters

Karol Serwin1, Kaja Scheibe1, Anna Urbanska1, Bogusz Aksak-Waj2, Magdalena Witak-Jedra1, Piotr Zabek1, Ewa Siwak1, Iwona Cieciarnia1, Pawel Jakubowski1, Monika Boscaga-Jaski1, Elżbieta Mularska1, Bartosz Szterela1, Aleksandra Szymczak1, Milosz Parczewski1
1Pomeranian Medical University, Szczecin, Poland, 2Medical University of Warsaw, Warsaw, Poland, 3Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland, 4Pomeranian Hospitals, Gdańsk, Poland, 5Jagiellonian University, Kraków, Poland, 6Medical University of Łódź, Łódź, Poland, 7Wincow Medical University, Wadowice, Poland

Background: Since February 2022, Poland has become a shelter for over 1.6 million Ukrainian refugees, a number comparable to the total migrant flow of the past decade. The HIV-1 A6 lineage, dominant in Eastern Europe, has become the most common variant in Poland in the recent years, previously dominated by subtype B infections. Phylogenetic analysis yields insights into transmission clusters and linkages, offering a deeper understanding of ongoing virus spread. In this study we explore the changing epidemic profile of the A6 lineage in Poland in the context of migration and refuge but also local transmissions.

Methods: We analyzed 1941 Polish HIV partial pol sequences, including 719 from Ukrainian-born individuals, supplemented with 11,807 location-annotated A6 sequences, collected up until November 2023. Phylogenetic inference and genetic distance-based clustering were employed to identify clusters and genetically linked individuals. Clusters were classified into singletons and dyads (≤2 sequences), networks (3–13 sequences), or large clusters (≥14 sequences). Further, in the Polish cohort, we delineated sequences from individuals born in Poland or Ukraine and analysed transmission routes within clusters.

Results: We identified six large clusters (n=855 sequences), 74 networks (n=353), and 590 singletons or dyads (n=733). In large clusters dominated internal Polish transmissions among men who have sex with men (MSM) accounting for the total of 41.9% of infections with known transmission route. There was also a marked rise in new, late diagnosed (lymphocyte count at baseline < 350 cells/μL) cases was observed among Ukrainian male (13.6%) and female (13.3%) migrants since the beginning of 2022, representing new introductions of the virus into mainland Poland, primarily as singletons and dyads but without further spread into larger clusters. The most common international linkages were observed from Ukrainian migrants diagnosed before 2022 to individuals born in Poland (35.0% of the normalized links).

Conclusion: The influx of war-displaced people from Ukraine notably fuels sub-subtype A6 infections in Poland but majority of introductions resulted in dead-end transmissions. On the other hand, large clusters define independently growing national epidemic with this variant in MSM. Presented data imply urgent need for targeted HIV testing and intervention programs among key populations. The figure, table, or graphic for this abstract has been removed.

1070 HIV Spread in Miami-Dade County

Antoine Chaillon1, Alan Wells2, Sanjay R. Mehta3, Susan J. Little1
1University of California San Diego, La Jolla, CA, USA, 2VA San Diego Healthcare System, La Jolla, CA, USA

Background: The Miami-Dade HIV epidemic is one of the largest and most active regional epidemics in the US. While predominantly an epidemic among men who have sex with men (MSM), there is significant diversity across ethnicity, race, risk group and geography. We characterized the dynamics of HIV transmission between neighborhoods and risk groups in the Miami-Dade region to inform the development of focused interventions.

Methods: A comprehensive public health dataset of 7274 HIV-1 subtype B partial pol sequences sampled from unique persons with HIV (PWH) between 2015-2020 was combined with a closely related background dataset of 4,250 publicly available sequences. A multistep phylogenetic approach was applied: (1) maximum likelihood phylogenetic inference to identify well-supported monophyletic clades of size ≥5 including ≥2 distinct Miami-Dade neighborhood groups (NBHDs), as defined by Florida Department of Health; (2) local clades were used to perform a discrete phylogeographic inference to evaluate transmission dynamics within NBHD (but not within a NBHD) after 2010. Additional metadata including stage of infection was also incorporated into the analysis to estimate migration between NBHDs to evaluate migration between risk groups.

Results: We identified 3,737 HIV sequences forming 315 transmission clusters of ≥5 sequences (range 5–130) that included PWH from at least two NBHDs. PWH belonging to these clades were predominantly male (76%), Hispanic (53%), and MSM (55%). Discrete phylogeographic analysis of identified clades suggested that the NBHD of Downtown/East Little Havana/Liberty City/Overtown (21% of all dispersal events, after 2010), Brownsville/Coral Gables/Coconut Grove (22%), and Aventura/Miami Beach (19%), were the major sources of viral flow to other NBHD in the Miami-Dade region (Fig. 1AB). Overall, heterosexuals and MSM accounted for 49.9% and 46.8%, respectively of putative sources of transmission linking different risk groups.

Conclusion: Phylodynamic analyses revealed complex HIV dispersal across NBHD in the Miami-Dade region with some key areas predominating as sources of viral dispersal in recent years. HIV transmissions between risk groups were nearly equally likely to arise from MSM and heterosexuals. These results highlight the role of the central east region in the geographic spread of HIV within Miami-Dade County. These analyses suggest earlier diagnosis and treatment of individuals in these neighborhoods may have outsized impacts across the region.

Figure: Relative contribution of NBHD and transmission risk groups to spread of HIV in Miami-Dade County. Inferred migration links included if adjusted Bayes Factor (BFadj) ≥ 3. A. Sankey plot representing the proportion of transition events from "in" each source to "to" recipient NBHD after 2010. B. Sankey plot representing proportion of transition events between risk groups.

1071 Genetic Diversity From Proviral DNA as a Proxy for Time Since HIV-1 Infection

Marius Zeeb1, Paul Frischknecht1, Karin Metzner2, Michael Huber2, Christine Leeman3, Julia Notter4, Andre Rauch5, Marcel Stöckle6, Alexandra Calmy7, Matthias Cavassini8, Enos Bernasconi8, Dominique Braun9, Haldur F. Günthard10, Roger Kooijas11, for the Swiss HIV Cohort Study

1University Hospital Zurich, Zurich, Switzerland, 2University of Zurich, Zurich, Switzerland, 3St Gallen Cantonal Hospital, St Gallen, Switzerland, 4University Hospital of Bern, Bern, Switzerland, 5University Hospital Basel, Basel, Switzerland, 6University Hospitals of Geneva, Geneva, Switzerland, 7Lausanne University Hospital, Lausanne, Switzerland, 8Ospedale Regionale di Lugano, Lugano, Switzerland

Background: Knowing the time since infection in people with HIV (PWH) is relevant for transmission epidemiology, HIV pathogenesis and for many research questions in general. As viral genetic diversity accumulates over time, in the absence of ART, diversity-based scores measured from ART-naïve viral RNA sequences have been shown to be predictive of the time since infection. However, the validity of this approach is unclear for proviral NGS sequences sampled on ART. A particular challenge is noise from APOBEC3G/F induced hypermutations.

Methods: We assessed the association between time since infection (date of infection until ART initiation) and viral genetic diversity calculated as the average pairwise diversity score from proviral pol, gag, and env NGS sequences. Samples were taken after ART initiation from PWH with exact infection dates known via a comprehensive assessment of the medical history. All samples were from PWH enrolled in the Swiss HIV Cohort Study or Zurich Primary HIV Infection Cohort study. We used linear regression to determine the R2 and mean absolute error (MAE) of genetic diversity as a proxy for time since infection. We used AUC-ROC to quantify infection recency prediction. Over all analyses we applied different hypermutation filtering thresholds on NGS read level to account for APOBEC3G/F induced 6- to A-mutations.

Results: We identified 261 PWH with accurate HIV infection dates and available NGS sequences for at least one gene (with >100 codon coverage) across all hypermutation thresholds (n=233 gag, 233 pol, 208 env). Among those, 146 PWH had full codon coverage (n=91 gag, 74 pol, 67 env). We found that proviral genetic diversity was predictive for the time since infection: average pairwise diversity scores calculated both with and without hypermutation filters were significantly associated with time since infection (p<5×10^-10). The R2 ranged from 6% for env without hypermutation filtering to 48% for pol with the...
strictest filter (Figure A). The MAE ranged from +/-1.01 to +/-1.77 years. Recent infection prediction showed an AUC of 0.92 (95% CI 0.86, 0.98) for pol with the strictest filter (Figure B).

**Conclusion:** This work shows the utility of genetic diversity measured from proviral sequences as a proxy for the time between ART initiation and infection. While genetic diversity measured in ART-naive viral RNA is more accurate, a good performance can be achieved with hypermutation filtering and hence allows to determine infection recency in PWH without a baseline RNA sequence.

![Image](https://via.placeholder.com/150)

**1072 The Impact of COVID-19 on HIV Mortality Trends in United States Black, White, and Hispanic Adults**

**Elizabeth B. Pathak, DaRel M. Barksdale, Mary Y. Masterson, Paul A. Burns**

**National Heart, Lung, and Blood Institute, Bethesda, MD, USA**

**Background:** U.S. racial and ethnic minorities disproportionately impacted by HIV have also been severely impacted by COVID-19. This study investigates whether COVID-19 led to excess HIV mortality, using rigorous methods which account for age, gender, and race/ethnicity disparities in pre-pandemic HIV mortality trends.

**Methods:** Death data from CDC WONDER were analyzed for all decedents with HIV listed as either underlying or contributing cause of death. Pre-pandemic trends in HIV mortality were quantified by fitting log-linear models to HIV death rates for the period 2010-2019. Separate models were fit for groups defined by age (25-44, 45-54, 55-64, 65-74, 75+), reported gender (male, female), and race/ethnicity (Black, Hispanic, white). Regression coefficients were used to derive the average annual percent change in HIV mortality from 2010-2019, and to calculate expected death counts and rates for 2020-2022. Regression-estimated expected death rates were then compared with observed rates, and the number of excess deaths calculated. Finally, the number of excess HIV deaths for 2020-2022 was compared to the number of HIV deaths for which COVID-19 was listed as a contributing or underlying cause of death.

**Results:** Across all age groups, Black men suffered the highest rates of HIV mortality (see Figure). Across all gender-race/ethnicity groups, HIV mortality declined significantly among young adults (25-44 and 45-54 years). However, there were no declines in HIV mortality for those aged >55 years (Whites and Hispanic women) or >65 years (Blacks and Hispanic men). Moreover, statistically significant increases in HIV mortality were observed for white men >55 years, and for Black women and Black men 65-74 years. During 2020-2022, there were 3,263 excess HIV deaths among all groups, and COVID-19 was mentioned on 73.2% of those death certificates. The percent of excess HIV deaths which listed COVID-19 as a contributing cause varied significantly by race/ethnicity, gender, and age.

**Conclusion:** Black and Hispanic adults have continued to experience disproportionately high HIV mortality during the COVID-19 pandemic. HIV mortality trends varied widely by age, with declines in young adults but no declines or increases in older adults. COVID-19 diagnosis may not have been listed for some excess HIV deaths, particularly among 25-44 year olds, Blacks, and Hispanics. Further investigation of direct SARS-CoV-2 infection effects vs. indirect pandemic effects in explaining observed excess HIV mortality is needed. The figure, table, or graph for this abstract has been removed.

![Image](https://via.placeholder.com/150)

**1073 COVID-19-Related Shutdowns and Viral Suppression in the US and Canada**


**1 The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2 Columbia University, New York, NY, USA, 3 Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4 Kaiser Permanente Mid-Atlantic States, Rockville, MD, USA, 5 Southern Alberta Clinic, Calgary, Canada, 6 Vanderbilt University, Nashville, TN, USA, 7 University of Washington, Seattle, WA, USA, 8 VA Connecticut Healthcare System, West Haven, CT, USA, 9 Emory University, Atlanta, GA, USA, 10 Kaiser Permanente Northern California, Oakland, CA, USA, 11 University of North Carolina at Chapel Hill, Chapel Hill, NC, USA**

**Background:** COVID-19 disrupted in-person HIV care across North America. We compared frequency and type of HIV care encounters and viral suppression among people with HIV (PWH) prior to, during, and after COVID-19 shutdowns in the largest cohort collaboration of PWH linked to care in the US and Canada.

**Methods:** Adult (≥18yo) PWH in 13 NA-ACCORD clinical cohorts newly engaged or re-engaged (if absent since 1 Sept 2018) in HIV care (>1 in-clinic or telemedicine [phone/video] visit, CD4 measure, or HIV RNA measure) during shutdowns (1 Mar 2020–31 May 2020) were compared with those in HIV care prior to the pandemic (1 Sept 2018–29 Feb 2020). Within each group, we described numbers of telemedicine visits and proportions of suppressed HIV RNA measures (<50 copies/mL) each month from Jan 2019-Dec 2021.

**Results:** There were 34,722 PWH in HIV care prior to the pandemic, of whom 51% received care during shutdowns, and 11% did not return to care as of 31 Dec 2021. There were 568 newly engaged (n=323, 57%) or re-engaged (n=245, 43%) PWH during shutdowns. Compared with those in prior care, those who newly engaged or re-engaged in care during shutdowns were younger (median=40.7 [30.9-51.8] vs. 49.7 [38.5-57.6] years), more likely to be Black (41% vs 38%), and a greater proportion had not initiated ART (20% vs. 2%, all p-values <0.01). Among PWH in care prior to the pandemic, telemedicine visits increased, and viral suppression was >80% during (n=9,159 viral load measures [VLs]) and after (n=81,994 VLs) shutdowns (Figure). Among those engaging or re-engaging in care during shutdowns, in-person visits were more common than telemedicine; viral suppression was 42% in April (n=131 VLs) and >85% in August 2020 (n=80 VLs). Among those in prior care, viral suppression remained stable, median time between care encounters were similar before and after (1 Jun 2020–31 Dec 2021) shutdowns (n=116 [40-182] vs. 109 [41-182] days, p<0.05), as was time between HIV RNA measures (182 [122-223] vs. 163 [105-203] days, p<0.05).

**Conclusion:** Among PWH newly engaged or re-engaging in care during the pandemic in the NA-ACCORD, >85% of HIV RNA measurements were suppressed 3 months after shutdowns ended. With the rapid scale-up of telemedicine, half of adults in HIV care prior to the pandemic connected to HIV care in March–May 2020, maintained a high proportion of viral suppression, and had similar frequency of HIV care encounters and viral load measures before and after shutdowns.

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**Ellen M. Tedaldi, Jingjiang Hou, Carl Armon, Jonathan Mahanek, Frank Paila, Gina Simoncini, Jack Fuhrer, Cynthia Mayer, Alexander C. Ewing, Kalliope Chaqariz, Kimberly Carlson, Jun Li, Kate Buchacz**

**1 Temple University, Philadelphia, PA, USA, 2’s Center for Aids Research, Kansas City, MO, USA, 3’s Northwestern University, Chicago, IL, USA, 4’s AIDS Healthcare Foundation, Philadelphia, PA, USA, 5’s State University of New York at Stonybrook, Stony Brook, NY, USA, 6’s Joseph C. Hemphill Comprehensive Research Institute, Tampa, FL, USA, 7’s Centers for Disease Control and Prevention, Atlanta, GA, USA**

**Background:** In 2020, the SARS-CoV-2 pandemic caused an unprecedented strain on the spectrum of services for persons living with HIV, including
This study assessed variation in viral load (VL) testing and outcomes related to the impact of telehealth use on HIV care outcomes is conflicting. There was a relative maintenance of viral suppression. Ongoing recovery patterns over the subsequent 2 years albeit with overall declines for in-person care. There was an increase in telemedicine visits per person-month from 0.001 in January 2020 to 0.16 in May 2020, before declining to near pre-pandemic levels in 2022 (Figure 1A). The average rate of in-person per-person-month decreased from 0.32±0.01 (mean±std) during 2010-2019 to 0.24±0.08 in 2020. The average rate of telemedicine visits per person-month was near zero during 2010-2019 and increased to 0.07±0.05 during 2020. In multivariable logistic regression models, persons with missing encounters were more likely to be male or have VL ≥200 copies/mL. For participants with ≥ 1 viral load test, the prevalence rate of detectable HIV viral load during 2020-2022 was closer to the rate from 2014-2019 (Figure 1B). The change in probability of viral suppression was not associated with participant’s age, sex, race/ethnicity, or insurance status.

Conclusion: In the HOPS, there were immediate changes in visit type in response to the early COVID-19 pandemic followed by a return to previous patterns over the subsequent 2 years albeit with overall declines for in-person visits. There was a relative maintenance of viral suppression. Ongoing recovery efforts to improve access to medical services and care retention.

Results: The annualized reported COVID-19 mortality rate was 131.3/100,000 in the Western Hemisphere starting from the first reported death to 12/31/2021. We adjusted for underreporting using three models developed by the Institute for Health Metrics and Evaluation (IHME), the World Health Organization (WHO), and the Economist. Age-adjusted analysis was performed to control for the association between COVID-19 mortality and older age. To account for additional country-level variations in mortality rates, we collected publicly available data on underlying medical conditions associated with higher risk for severe COVID-19 (identified by the Centers for Disease Control), as well as demographic and socioeconomic factors. Univariate and multivariate analyses were conducted to evaluate associations between each risk factor and adjusted COVID mortality rate.

Methods: Using data reported from each country’s health ministry, we examined COVID-19 mortality rates across countries and regions in the Western Hemisphere starting from the first reported death to 12/31/2021. To adjust for underreporting using three models developed by the Institute for Health Metrics and Evaluation (IHME), the World Health Organization (WHO), and the Economist. Age-adjusted analysis was performed to control for the association between COVID-19 mortality and older age. To account for additional country-level variations in mortality rates, we collected publicly available data on underlying medical conditions associated with higher risk for severe COVID-19 (identified by the Centers for Disease Control), as well as demographic and socioeconomic factors. Univariate and multivariate analyses were conducted to evaluate associations between each risk factor and adjusted COVID mortality rate.
(Figure). After adjusting for underreporting and age, the countries with the highest mortality rates were Bolivia, Peru, Ecuador, and Mexico. In multivariate regression analyses, GDP emerged as the only significant predictor of COVID-19 mortality.

**Conclusion**: Countries in Andean Latin America consistently had the highest COVID-19 mortality rates, even after accounting for underreporting and age difference. Country-level differences were largely attributed to socioeconomic status, with lower- and middle-income countries having the highest rates of COVID-19 mortality in the Western Hemisphere.

**1077 What’s in a Wave: Using COVID-19 Data to Explore the Definition of Epidemic Waves**
*Joshua Smith-Sreen, Jorge Ledesma, Mark Lurie*
Brown University, Providence, RI, USA

**Background**: Objectively defining and classifying epidemic or pandemic waves is critical in providing opportunities for timely resource allocation. However, there is no consensus about what composes a pandemic wave despite proposed definitions in the literature. This analysis aimed to identify, apply and characterize wave definition approaches using COVID-19 case data to build towards standardized definitions for improving pandemic preparedness and response.

**Methods**: We obtained daily United States (US) case data from February 2020 to March 2022 from the Johns Hopkins COVID-19 data repository. We identified three major definitions of epidemic waves by scoping review. The “eR approach” defined waves as periods where the effective reproduction number (eR) was greater than 1 for at least 14 days. The “fold approach” defined waves as periods where the weekly case rate increased by at least one-fold followed by a decrease by at least one-fold. The “threshold approach” defined waves as periods where the weekly case rate per 100,000 population surpassed 49.99, the US CDC threshold for moderate community transmission. We then compared wave characteristics across definitions.

**Results**: The eR approach generated 5 waves with an average length of time between waves of 79 (IQR 47–90) days and average wave duration of 65 (56–73) days. The fold approach generated 10 waves with 25 (7–44) days between waves and average wave duration of 47 (29–54) days. The threshold method produced 2 waves with 62 days between the two waves and average wave duration of 276 (251–301) days. The in-wave average daily case rates per 100,000 population were 24.2 (95% CI 20.9–28.1), 19.7 (17.2–22.5), and 20.4 (18.6–22.3) for the eR approach, fold approach, and threshold approach, respectively.

**Conclusion**: This analysis provides novel characterization of various approaches to epidemic wave definition. The fold approach produced the greatest number of waves, likely due to its sensitivity to weekly changes in case rates. The threshold approach produced the highest in-wave average case rate and lowest between-wave average case rate, indicating it may not adequately capture periods of infection. These findings have public health implications, as overestimating waves may trigger preemptive allocation of limited health resources, and underestimating may result in a reactionary response with poor targeting of treatment and prevention. Further application of the definitions across countries and diseases are needed to build towards consensus.

**1078 National HIV Testing Trends and Sociodemographic Correlates in Black Cisgender or Transgender Women**
*Xinyi Li1, Yijin Xiang2, Jincong Q. Freeman3, Yong G. Lee4*
1George Washington University, Washington, DC, USA, 2University of Southern California, Los Angeles, CA, USA, 3University of Chicago, Chicago, IL, USA, 4Rutgers University, Newark, NJ, USA

**Background**: Black cisgender and transgender women are disproportionately impacted by HIV in the United States. Having high-risk behaviors related to HIV but not receiving HIV testing could lead to delayed diagnosis and treatment in this population. We investigated the trend of HIV testing and associated sociodemographic characteristics among Black cisgender and transgender women at risk for HIV.

**Methods**: We analyzed data from the 2016–2020 Behavioral Risk Factor Surveillance System. Respondents who self-identified as Black, cisgender or transgender women, and reported high-risk behaviors were included. Variables examined in the analysis included ever been tested for HIV, age, education level, income, healthcare coverage, and geographic region. Unweighted counts and weighted percentages were used to describe study population’s characteristics. Annual percent change (APC) and p-value were calculated to examine the trend of ever having been tested for HIV over 5 years, using the Joinpoint trend analysis software. The associations between sociodemographic characteristics and ever having HIV testing were assessed using multivariable logistic regression, controlling for explanatory variables with a p<0.05. Adjusted odds ratio (aOR) and 95% confidence intervals (95% CI) were reported.

**Results**: Among 3,509 (weighted n= 4,129,231) Black cisgender and transgender women (35 were excluded due to missing HIV testing status), 2,837 (83.1%) had ever been tested for HIV, and 647 (16.9%) had not. We observed a slight decrease in the percentages of HIV testing over 5 years, from 83.8% in 2016 to 79.2% in 2020 (APC = -1.29, p=0.5420). Black cisgender and transgender women aged 18-34 years (aOR: 0.47 , 95% CI: 0.33-0.65) or ≥55 years (aOR: 0.18, 95% CI: 0.11-0.31) were less likely than women aged 35-54 years to get tested for HIV. Additionally, Black cisgender and transgender women with less than high school (aOR: 0.61, 95% CI: 0.38-0.99) or some post-high school (aOR: 0.63, 95% CI: 0.46-0.86) education were less likely to get tested for HIV compared to those with at least a college degree.

**Conclusion**: In this nationally representative sample of Black cisgender and transgender women, more than one in 6 with high-risk behaviors had not been tested for HIV, and this trend remained stable over the 5-year period. To address the missed opportunities in HIV testing, HIV prevention programs might consider targeting 18-34 years old and ≥55 years Black women and those with a lower level of education.

**1079 Seroprevalence of Mpox IgG Antibodies in a Cohort of PLWH in Rome During the 2022 Outbreak**
*Pierluigi Francesco Salvo1, Rebecca Jo Steiner1, Damiano Farinacci2, Valentina Iannone1, Francesco Lamanna1, Rosa Anna Passerotti1, Alberto Borghetti1, Simona Di Giambenedetto1, Francesca Lombardit1*
1Catholic University of the Sacred Heart, Rome, Italy, 2IRCCS Fondazione Policlinico Universitario Agostino Gemelli, Rome, Italy

**Background**: The 2022 outbreak of MPXV has unveiled atypical epidemiological and clinical features, setting it apart from previous outbreaks.
On May 10th 2023 the WHO declared that MPXV is no longer a public health emergency of international concern. It was also emphasized that all countries worldwide should integrate MPXV prevention and care into national health programs to avoid future spreads. The clinical presentation of MPXV can range from mild to severe and mortality rates can vary. The high incidence rate further suggests that individuals who are infected but clinically asymptomatic may play an important role in the transmission of the virus. More research is needed to ascertain whether HIV can directly influence the clinical presentation in individuals affected by MPXV, focusing on the possibility of an asymptomatic course in this group of individuals. We undertook this study to assess the seroprevalence of IgG anti-MPV in a cohort of PLWH with no reported symptoms consistent with a diagnosis of MPXV, to try to analyze the actual size of the phenomenon of asymptomatic infections in clinical practice.

**Methods:** From October 2022 to February 2023, we serially collected serum samples from PLWH attending our outpatient clinic for their routine analysis. IgG against MPV have been assessed on stored cryopreserved serum samples with an ELISA. No significant cross-reactivity or interference between anti-MPV IgG and analogues was reported. For the purpose of this study, only people with no previous reported vaccine against smallpox or MPXV nor previous clinical manifestations consistent with an MPXV diagnosis were included.

**Results:** A total of 104 PLWH were tested. 19 participants reported a previous vaccination against smallpox, 1 participant reported a previously confirmed diagnosis of MPXV. All the other 84 participants denied previous vaccination, infection or clinical manifestations consistent with MPXV infection. Our analysis revealed 6 patients who tested IgG positive for MPX. Seroprevalence was equal to 7.1%. Demographical and viro-immunological characteristics of the entire population and PLWH who tested IgG positive are shown in Table 1.

**Conclusion:** Our findings from this setting showed a mildly high IgG MPXV prevalence among PLWH with no previous clinical manifestations, suggesting the possibility of an asymptomatic course of the MPXV infection. Early detection and appropriate management of MPXV infected people are of utmost importance for global public health and appropriate clinical management.

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**1080 Impact of COVID-19 Pandemic on HIV Testing Among MSM: An Interrupted Time Series Analysis**

**Emily J. Green,** Lamia Khan, Jamieson T. Jann, Marjan Javanbakht, Risa Flynn

Los Angeles LGBT Center, Los Angeles, CA, USA, University of California Los Angeles, Los Angeles, CA, USA

**Background:** We have examined the effects of COVID-19 pandemic on HIV testing across substantial observation time among a large sample of gay, bisexual and other men who have sex with men (GBMSM), a priority population disproportionately impacted by HIV. We describe the extent to which COVID-19 pandemic-related disruptions to health clinic services impacted the level of HIV testing among sexual minority men in Los Angeles.

**Methods:** Data comes from the Los Angeles LGBT Center’s health services program, one of the nation’s largest Federally Qualified Health Centers providing health services to LGBTQ+ individuals. We include GBMSM who are 15 years or older. The primary outcome measure is the average number of HIV tests per week. Interrupted time series analyses were used to model and test changes in HIV testing across three phases of the pandemic: a pre-pandemic phase from March 1, 2018 to March 15, 2020, a pandemic phase from March 16, 2020 to February 28, 2022, and an endemic phase, from March 1, 2022 to August 31, 2023, which signifies the full return of Center services. Weekly HIV testing is estimated overall and by race/ethnicity.

**Results:** The sample included 25,844 unique participants (n=95,328 visits); 8% were Black/African American, 34% Hispanic/Latinx, 39% white, 8% Asian/Pacific Islander, and 11% some other race/ethnicity. Most participants were between 25-39 years old (48%). The average number of tests conducted weekly across the pre-pandemic, pandemic, and endemic phases are 321, 231, and 288, respectively. There was nearly a 50% reduction in the level of HIV testing conducted weekly after the transition from the pre-pandemic to pandemic phase (Incident Rate Ratio (IRR): 0.53, 95%CI: 0.47, 0.60). The number of HIV tests decreased slightly going from the pandemic to endemic phase (IRR = 0.80, 95%CI: 0.7, 0.92) and HIV testing did not return to pre-pandemic levels (IRR = 0.77, 95%CI: 0.64, 0.93). Similar trends were observed when stratified by race/ethnicity, however greater decreases in number of tests during the pandemic phase were greater for White and Hispanic/Latinx participants compared to Black/African American and Asian/Pacific Islander participants (Figure).

**Conclusion:** The overall reduction of HIV testing levels compared to pre-pandemic suggests a new strategy needs to be developed to understand which participants are not returning for testing and why as well as what additional efforts are needed to increase the reach of these necessary preventive services.
1082 WITHDRAWN

1083 Test-All Model for SARS-CoV-2 Testing Is More Cost-Effective Than Screen & Test in Kenya & Cameroon

Mario J. Songane1, Boris Tchounga1, Rose Masaba2, Tatiana Djiekussi1, James Ndimbii2, Carolyn Mvancha-Kwasa1, Emilienne Epe1, Anne Bissel1, Aida Famanerham1, Laura Guay1, Rhoderick Macheke1, Nilesh Bhath1, Appollinaire Tiam1, Suchant Mukherjee2, Elizabeth Glaser Pediatric AIDS Foundation, Maputa, Mozambique, Elizabeth Glaser Pediatric AIDS Foundation, Discotheque, Cameroon, 1Elizabeth Glaser Pediatric AIDS Foundation, Maputa, Mozambique, 2Elizabeth Glaser Pediatric AIDS Foundation, Discotheque, Cameroon, 3University College London, London, United Kingdom, 4University of North Carolina Project–Malawi, Lilongwe, Malawi, 5University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Cameroon and Kenya currently use a SARS-CoV-2 “screen and test” (ST) model, offering testing if clients have COVID-19-like symptoms or were exposed. In a randomized control trial comparing two testing models, the test-all (TA) model (testing offered regardless of screening outcome) identified more SARS-CoV-2 positive cases than the ST model. It is important to determine costs and cost-effectiveness associated with implementation of the TA model compared to the ST model prior to expansion.

Methods: The total costs of implementing TA and ST in Cameroon and Kenya were estimated from a health systems perspective using a micro-costing method. The cost per client tested (PCT) and tested positive (PCTP) were estimated by dividing the total cost of each model by the number of clients tested and tested positive, respectively. A decision tree designed in TreeAge and cost-effectiveness acceptability curve were used to compare the cost-effectiveness of the two models. One-way sensitivity and probabilistic sensitivity analyses were used to assess the effect of uncertainties in key parameters on costs per client and cost-effectiveness, respectively.

Results: In Cameroon, the total cost of TA was $141,942 and ST was $48,020. In TA model, the cost PCT was US$8 and the PCTP for SARS-CoV-2 was $509, whereas in the ST model the cost PCT was $25 and the cost PCTP was $728. The biggest expenditure in the TA model was SARS-CoV-2 antigen rapid diagnostic tests (Ag-RDT), 61% ($86,833), and for ST was personnel, 39% ($18,592). In Kenya, the total cost of TA was $39,264 and for ST was $27,500. In the TA model the cost PCT for SARS-CoV-2 was $13 and PCTP was $1,190, whereas in the ST model the cost PCT for SARS-CoV-2 was $125 and PCTP was $1,250. In both models in Kenya, the biggest expenditure was personnel, which corresponded to 45% ($17,696) in TA and 56% ($15,267) in the ST model. In both countries, TA model was more cost-effective.

Conclusion: With the current global efforts to lower the price of SARS-CoV-2 Ag-RDT, TA model is likely to be more cost-effective. The widespread implementation of TA model, as done in this project, would help identify priority areas for vaccination and individuals with SARS-CoV-2 infection early for treatment/quarantine. When budgeting for expansion of TA model, the estimated population size, costs of SARS-CoV-2 Ag-RDT, and personnel must be accurate, since they were shown in the sensitivity analysis to have the biggest impact on costs per client and cost-effectiveness.

1084 Validation and Acceptability of SARS-CoV-2 Loop-Mediated Isothermal Amplification Test in Malawi

Maggie Nyirenda Nyang’wa1, Mercy Kamdolazi1, Harry Meleke1, Vincent Samuel Phiri1, Thomas Waterfield1, Nedron Bondera1, Maganizo B.1, Chagomerana2, James McKenna3, Thandie Mwalukalo4, Chiroma Mseufula5, Tonney Nyirenda6, Derek Fairley1, Mima Hasseinepour1, for the SARS LAMP Group1, 1University of North Carolina Project–Malawi, Lilongwe, Malawi, 2Queen’s University Belfast, Belfast, United Kingdom, 3Queen Elizabeth Central Hospital, Blantyre, Malawi, 4Umbrella Organization for Health Systems Improvement, Lilongwe, Malawi, 5University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Real-time reverse-transcription Polymerase-Chain-Reaction (RT-PCR) is the gold-standard diagnostic test to confirm SARS-CoV-2 infection however RT-PCR is expensive requiring specialist laboratories. Alternatively, optimised nucleic-acid tests such as SARS-CoV-2 reverse-transcribe-chain-loop-mediated-isothermal-amplification (SARS-LAMP) could minimise costs and enable testing in settings without specialist laboratories. We evaluated the diagnostic test accuracy (DTA) (sensitivity detecting cycle threshold (CT) values <30; specificity >95%), acceptability and user-friendliness of SARS-LAMP test.

Methods: Following optimisation of SARS-LAMP, a DTA study was conducted at Queen Elizabeth–Central–Hospital (QECH) COVID-19 testing centre in Southern Malawi between September 2021-2022. Nasopharyngeal swabs were collected and tested for SARS-CoV-2 by laboratory technicians using SARS-LAMP at Kamuzu University-of–Health–Sciences (KUTH) laboratory. The reference standard test was defined as RT-PCR or rapid-antigen–testing (RAT) for SARS-CoV-2 performed using any commercial platform at QECH. We calculated sensitivity and specificity of SARS-LAMP compared to existing commercial tests. SARS-LAMP’s user-friendliness by laboratory technicians (n=4) and acceptability by suspected cases of COVID-19 and contacts (n=68) were assessed using semi-structured interviews. Likert scales were analysed using summation analysis. Major themes and subthemes were identified.

Results: 105 retrospective nasopharyngeal swabs samples were analysed during optimisation and SARS-LAMP had sensitivity-73.7% (95% CI:65.3%–89.2%); specificity-100% (95% CI:100.0%–100.0%). The prospective DTA study recruited 450 participants using convenience sampling. Median age-37 years (IQR:28-57 years); 248/450(55%)-male, 61/450(13.6%)- contacts, 259/450(58%)—vaccinated. 233/450 (52%) had comorbidities, 192/450 (43%) were SARS-CoV-2 positive, 5/192(3%) were admitted to ICU,
Estimation of Recent HIV Infections in Japan Using a Novel Testing Algorithm With Chronological Tree

Teichiro Shinoh, Shuzo Matsuhashi, Tadaaki Kikuchi, Machiko Otani, Kazuhisa Yoshimura, Wataru Sugihara, for the Japanese Drug Resistance HIV-1 Surveillance Network

1 National Center for Global Health and Medicine, Tokyo, Japan
2 Yamamoto University, Kofu, Japan
3 National Institute of Infectious Diseases, Tokyo, Japan
4 Tokyo Metropolitan Institute of Public Health, Tokyo, Japan

Background: In Japan, approximately 30% of newly diagnosed individuals with HIV are identified after the onset of AIDS symptoms, suggesting that the diagnosis of people living with HIV is delayed. The lack of a serological avidity assay in Japan’s surveillance system makes it difficult to accurately estimate HIV incidence. Therefore, we developed a recent infection testing algorithm (RITA) using the viral sequence-based time of the most recent common ancestor (tMRCA), based on information from our drug resistance surveillance instead of the avidity assay, and investigated the recency of some major domestic transmission clusters (dTCs) in Japan.

Methods: The Japanese HIV Drug Resistance Surveillance Network has monitored the dTC dynamics of newly diagnosed HIV-1 cases in Japan using our search program for HIV nationwide clusters by sequence (SPHINCS), which stored 10,445 newly diagnosed cases between 2003 and 2022. We extracted the sequences and clinical information of three subtype B and one CRF01_AE dTC prevalent during 2018–2022 and singleton cases from SPHINCS and inferred their chronological phylogeny using BEAST1. Based on the tMRCA between neighboring cases in the phylogeny, CD4+ T-cell count, viral copy number, and possibility of an acute infection, we constructed a RITA estimating the probability of recent or late diagnosis of each case.

Results: The cases available for our RITA were 529 in 2018, which decreased to 441 in 2020 and halved to 332 in 2022. All four targeted dTCs had men who have sex with men as a major risk factor. The recency of major dTCs in 2018–2022 ranged from 2.4 to 28.9%, and the recency of singleton cases was 10.0%. The overall estimated recency was 20.3%, assuming that other dTC affiliation cases were similar to major dTCs. The annual recency for each dTC was mostly less than 25%; however, it was >30% in 2018 for B-T2C1 and in 2018–2019 for AE-T2C, when outbreaks were observed in a region. The annual recency estimates for the total population consistently decreased, from 24.3% in 2018 to 15.7% in 2022.

Conclusion: Our results showed that before 2019, HIV testing in Japan rapidly diagnosed ongoing HIV transmission in some local risk populations, but this was not the case after 2020. As the number of positive cases decreased, HIV testing opportunities also decreased during the COVID-19 pandemic, which may have been a barrier to early detection. The decline in diagnoses of recent infections suggests a problem with the testing system in Japan.

Rapid Diagnosis of HIV Infection and Initiation of Antiretroviral Treatment

Monica Mantilla Suarez, Leonardo Arenal, Hector Mueses

1 Virrey Solís IPS, Bogotá, Colombia
2 Corporación de Lucha Contra el Sida, Santiago de Cali, Colombia

Background: In Colombia, 39% of HIV infections are identified at stage 3, and the mean delay for starting antiretroviral treatment (ART) is 35 days after confirmed diagnosis. It is well known that once a diagnosis is established, the sooner ART is initiated, the higher the benefits in terms of therapeutic goals and reduction of transmission. There are several barriers to getting tested in real life: Medical prescription, informed consent might be requested, and administrative burden, among others. A potential solution could be a point of care test (POCT), which has been only implemented for pregnant women and in some community centers.

Methods: An open-label randomized clinical trial was initiated comparing a group who received POCT vs. standard diagnostic protocol in a Medellin (CO) clinic. Patients with an HIV test order were randomized via telephone to receive POCT or standard diagnostic protocol. POCT included HIV screening and other tests (Syphilis, Hepatitis B, Hepatitis C, CD4 count and HIV viral load). Recruitment stopped when 50 diagnoses of HIV have been done in the POCT arm.

Results: From Jan 31, 2022, and Jan 31 2023, from a universe of 17,859 HIV tests, 327 new infections were detected. Fifty patients were identified via POCT (per protocol) and 277 with the standard diagnostic approach. The time from test order to sampling was <48h in 95% of POCT vs. 15% in standard protocol (p<0.05). 100% of patients with POCT got a viral load measured in <8 days after the first positive test vs. 0% in the standard protocol. The mean time to initiation of ART after the first diagnostic test was 20 days in POCT vs. 52 days in standard protocol (p<0.05).
1088 Targeted Outreach to Increase Linkage to Preventative Services for Patients Tested for Mpox

Hannah Blanchard1, Helen King1, Kristin Alvarez2, Ank Nijhawan3, Virali Soni1
1University of Texas Southwestern, Dallas, TX, USA, 2Hanklpnd Health and Hospital Systems, Dallas, TX, USA

Background: Early in the Mpox outbreak, patients presenting for Mpox testing often did not receive comprehensive sexually transmitted infection (STI) screening upon presentation. Increased coordination of care for linkage to comprehensive preventative services such as HIV pre-exposure prophylaxis (PrEP) and other STI screening is critical to improving both individual and public health outcomes. We hypothesized that targeted outreach to those tested for Mpox would increase rates of PrEP counselling and follow-up STI screening.

Methods: A retrospective chart review was conducted of individuals tested for Mpox between June 2022 and March 2023 at a large county health system in Dallas, Texas. This study assessed the impact of targeted outreach to Mpox-tested persons to promote comprehensive HIV and STI testing and linkage to preventative care services like PrEP. Chi-square (χ²) test was used for categorical variable analysis.

Results: A total of 414 individuals were tested for Mpox with 203 PCR-confirmed cases. At the time of Mpox testing, 238/414 (57.6%) individuals were previously diagnosed with HIV. An additional 76/76 (32.7%) were screened for HIV, and six new cases of HIV were identified at the time of Mpox testing. Thirty-three percent (136/414) of patients were tested for other STIs (chlamydia, gonorrhea, or syphilis) at the same time as Mpox testing with 45 new cases of STIs identified. As part of targeted outreach following the initial presentation for Mpox testing, 94/414 (22.9%) individuals were contacted. Patients who were eligible for HIV screening that received targeted outreach were more likely to be tested for HIV (10/26 [38.4%] vs 58/94 [62%]) (p<0.001). More individuals eligible for PrEP in the outreach group were counselled on starting PrEP than in the non-outreach group, (14/26 [53.8%] vs 8/144 [5.6%]) (p=0.0016). Similarly, more individuals that received outreach underwent additional STI testing compared to those without outreach (58/94 [62%] vs. 109/320 [34%]) (p<0.001). More individuals eligible for HIV testing often did not receive comprehensive sexually transmitted infection (STI) screening upon presentation. Increased coordination of care for linkage to comprehensive preventative services such as HIV pre-exposure prophylaxis (PrEP) and other STI screening is critical to improving both individual and public health outcomes. We hypothesized that targeted outreach to those tested for Mpox would increase rates of PrEP counselling and follow-up STI screening.

Conclusion: Targeted outreach increased screening for HIV and other STIs and counselling for PrEP among patients presenting for Mpox testing. Strategies to increase and standardize linkage to comprehensive preventative services are needed to reduce co-infections of Mpox, HIV and other STIs, and promote public health.

1089 Detection of Diverse HIV Strains by the m-PIMA™ HIV-1/2 Detect Point-of-Care Assay

Mark C. Anderson1, Lara I. Teodoro2, Fiona Harley3, Eduardo Almaraz, Carolyn Strobel1, Barbara Harris1, Todd V. Meyer1, Mary Rodgers1, Gavin Ciholtery1
Abbott Labs, Abbott Park, IL, USA

Background: HIV displays exceptionally high virus diversity that can impact detection by diagnostic assays which fundamentally rely on sequence conservation. Therefore, it is important to confirm their performance on the breadth of known HIV strains. We tested the m-PIMA™ HIV-1/2 Detect point-of-care (POC) assay against a diverse HIV panel consisting of group M and N, group O, Circulating Recombinant Forms (CRF), Unique Recombinant Forms (URF), and HIV-2. In silico inclusivity analysis of HIV-1 and HIV-2 sequences from NCBI was performed to predict m-PIMA detection to an even broader range of circulating strains.

Methods: We included 65,276 hospitalizations of PWUD at across 11 hospitals. Eleven hospital sites were included in the study. Nine established a cohort of inpatient encounters from 1/1/2020 to 4/1/2022 tied to the presence of CD-10 drug use diagnosis codes (Table 1). Two sites (CT and TX) identified inpatient cohorts from the same study period using Addiction Medicine consults. The unit of analysis was hospitalization. Data collected included: the number of hospitalizations, HIV antigen/antibody tests, HCV antibody tests, and consent policies, where consent requires either written or oral patient approval. The impact of state consent requirements on screening was analyzed using a Student's t-test comparing hospitals with and without these mandates.

Results: Across the cohort m-PIMA™ HIV-1/2 Detect test detected 262/274 (96.4%) samples. Mean (min-max) VL of the panel was 3.93 (2.18-6.14) log copies/mL. Among samples with HIV VL > 1000 copies/mL (VL > limit of detection of the m-PIMA™ HIV-1/2 Detect) 219/222 (98.6%) were detected. At least one panel member from each subtype/CRF and all URFs were detected. In silico analysis predicted that m-PIMA™ HIV-1/2 Detect would detect the majority of HIV-1 and HIV-2 strains. These data indicate that this assay can detect the full range of HIV viral diversity.

Conclusion: This study demonstrates the benefits of POC vs. standard protocol, reducing by more than 60% the time between confirmed diagnosis and initiation of ART. There are still opportunities for improvement because an immediate start of ART for patients from the POC could have happened if there were no additional administrative hurdles, such as a low opportunity for a timely doctor’s appointment.

1090 HIV & HCV Screen Rates for Hospitalized PWUD Are Heterogeneous & Suboptimal Across 11 US Hospitals

Leo K. Westgard1, Taisuke Sato2, Finlay Pitcher3, Emily D. Grussing3, Jessica P. Ridgway4, Ayesha Appa5, Jaimie P. Meyer6, Uriel R. Felsen7, Luara Marks8, Kimna Thakkar9, Brian T. Montague2, Ank Nijhawan10, William S. Bradford9, Ellen Eaton9, Alysse G. Wurcell7, for the PWUD Care Workgroup (PCW)
1Tufts Medical Center, Boston, MA, USA, 2University of Vermont, Burlington, VT, USA, 3Tufts University, Boston, MA, USA, 4University of Chicago, Chicago, IL, USA, 5University of California San Francisco, San Francisco, CA, USA, 6York University, New Haven, CT, USA, 7Albert Einstein College of Medicine, Bronx, NY, USA, 8Washington University in St. Louis, St. Louis, MO, USA, 9Maine Medical Center, Portland, ME, USA, 10University of Colorado Anschutz Medical Campus, Aurora, CO, USA, 11University of Texas Southwestern Medical Center, Dallas, TX, USA, 12University of Alabama at Birmingham, Birmingham, AL, USA

Background: To end the HIV and HCV epidemics, people who use drugs (PWUD) need more robust opportunities for HIV and hepatitis C virus (HCV) testing, confirmation of infection and linkage to care. While inpatient hospitalizations are an essential opportunity to test PWUD for HIV and HCV there is limited research on rates of inpatient testing for HIV and HCV among PWUD and no data comparing testing rates between hospitals or parts of the country. This primary aim of this study is to quantify aggregate testing rates across a cohort of U.S. hospitals and hospital systems. Secondarily, we aim to explore how HIV consent requirements impact testing rates.

Methods: Eleven hospital sites were included in the study. Nine established a cohort of inpatient encounters from 1/1/2020 to 4/1/2022 tied to the presence of CD-10 drug use diagnosis codes (Table 1). Two sites (CT and TX) identified inpatient cohorts from the same study period using Addiction Medicine consults. The unit of analysis was hospitalization. Data collected included: the number of hospitalizations, HIV antigen/antibody tests, HCV antibody tests, and consent policies, where consent requires either written or oral patient approval. The impact of state consent requirements on screening was analyzed using a Student's t-test comparing hospitals with and without these mandates.

Results: We included 65,276 hospitalizations of PWUD at across 11 hospitals. Sites had an average HIV screening rate of 40.08% (SD = 22.29%) and an average HCV Ab screening rate of 31.58% (SD = 15.14%), with widespread heterogeneity in screening rates across facilities. HIV screening rates did not significantly differ between states that require consent and those that did not (p-value = 0.389). Average test positivity across hospitals was 4.5% for HIV tests and 41.4% for HCV tests.

Conclusion: In a study of 11 hospitals and hospital systems across the U.S., we found suboptimal HIV and HCV testing rates during inpatient encounters for PWUD. Testing rates for HCV were lower than those for HIV, with widespread heterogeneity across hospitals, regardless of consent requirements. Hospitalizations are a missed opportunity to offer HIV and HCV testing. As treatment (HIV) and cure (HCV) are necessary to end these epidemics, understanding and overcoming barriers to HIV and HCV testing need to be prioritized. The figure, table, or graphic for this abstract has been removed.
1092 Declines in HIV Testing and Diagnoses: Unanticipated Consequences of the Trump Adm 2019 Title X Policy

Daniel S. Rosenbloom,1 Brad R. Evans,1 Brian Squadrone,1 Jennifer Nguyen,1 Livio Azzoni,1 Erin Coppola,1 Guoxin Wu,1 Jill W. Maxwell,1 Jessicamarie Morris,1 Kenneth Lynn,1 Paul Zuck,1 Pablo Tebas,1 Karam Mounzer,1 Luis J. Montaner,1 Bonnie Howell2

1Merck Research Laboratories, San Diego, CA, USA, 2Title X Family Planning Program (TPP), Office of Population Affairs, Office of the Assistant Secretary for Planning and Evaluation, Department of Health and Human Services

Background: In 2019 the Trump administration instituted a regulation (hereinafter the Policy) which required that all Title X funded family planning clinics provide 433,115 fewer HIV tests and diagnosed 2.3% fewer HIV cases than low exposed regions post-Policy. Interaction models showed HIV testing in high exposure regions declined significantly compared to low exposure regions from pre- to post- Policy (β -69,626 95% CI [-108,893, -30,359], p<.01) and Title X clinics in high exposure regions identified a significantly smaller proportion of the region’s total HIV cases compared to low exposure regions from pre-Policy (β -0.04, 95% CI [-0.07, 0.00], p=.05).

Conclusion: The 2019 Policy had a notable negative effect on HIV testing and diagnoses in the Title X program. These results extend the known negative health consequences of the Policy to include HIV-related outcomes. Established sexual and reproductive health providers in the Title X program are a key provider of HIV services; anti-abortion policies that endanger the Title X network also threaten to weaken the U.S. HIV response.

1093 HIV Outcomes Among Partners Reached by Phone vs In-Person for Assisted Partner Services in Kenya

Unmesha Roy Paladhi1, Edward Kariruth1, George Otieno2, James P. Hughes3, Harison Lagat1, Monisha Sharma1, Sarah Masyuko1, Paul Macharia1, Rose Bosire1, Mary Mugambi1, Carey Farquhar1, David Katz1

1University of Washington, Seattle, WA, USA, 2PATH, Nairobi, Kenya, 3PATH, Kisumu, Kenya, 4Kenyatta National Hospital, Nairobi, Kenya, 5Kenyatta Medical Research Institute, Nairobi, Kenya, 6Ministry of Health, Nairobi, Kenya

Background: Assisted partner services (APS) are an effective strategy for identifying and testing people with undiagnosed HIV and traditionally conducted primarily by phone with in-person contact for those unreachable by phone. However, less is known about the characteristics or HIV outcomes of those reached by different methods of contact.

Methods: We analyzed data from 31 facilities in Kenya providing APS to female index clients newly diagnosed with HIV, their male partners, and female partners of those men testing newly HIV-positive. APS providers attempted to contact partners using phone first if a number was available and, if unsuccessful after three phone calls, traced partners in-person in the community. Using log-linear mixed models, we estimated relative risks (RR) between phone and in-person contact for different characteristics or HIV outcomes of those reached by different methods of contact.

Results: From May 2018-March 2020, 2534 female index clients and 7614 male partners, of whom 772 (10.1%) tested positive and named an additional 4956 non-index female partners. Overall, we reached 11,912 (94.7%) partners, 5179 (43.5%) via phone and 6733 (56.5%) in-person. Among reached partners, being male (RR:1.25, 95% Confidence Interval [CI]:1.17-1.35) and completing secondary education or higher (RR:1.22, 95%CI:1.09-1.36) was associated with successful contact by phone. Of the 11,912 partners eligible for testing (reached by APS and no prior HIV diagnosis), 99.7% tested and 11.2% first-time tested. Of those reached by phone, 32% (1,280) of all Title X funded sites left the program. This exodus led to declines in Title X client numbers and contraception provision, however the Policy’s impact on HIV testing and diagnoses has yet to be examined.

Conclusion: In an APS program that reached 94% of elicited partners, fewer than half were successfully contacted by phone only. Men and those with higher education were more likely to be reached by phone, and partners receiving a new HIV diagnosis were more likely to be contacted in-person. Although phone-based tracing may reduce resources required for APS, a combined phone and in-person approach is likely essential to maintain a successful and equitable program.
1095  Community Network Driven COVID-19 Testing of Vulnerable Populations in the Central US: A RADx-UP RCT
En-Ling Wu1, Xiaquan Zhao2, Makenna Meyers1, Ellen Almior3, Gijar Payne4, Kavita Bhavan5, Nickolas Zaller1, Jerome Montgomery1, Anna Horton1, Russell Brewer1, Michelle Johns6, Matthew Aalasma7, Amelia Knopp1, Faye Taxman1, John Schneider1, for the C3 Investigators
1University of Chicago, Chicago, IL, USA, 2George Mason University, Fairfax, VA, USA, 3Capital Area Reentry Program Inc, Baton Rouge, LA, USA, 4University of Texas Southwestern, Dallas, TX, USA, 5University of Arkansas for Medical Sciences, Little Rock, AR, USA, 6Project VIDA, Chicago, IL, USA, 7NORC at the University of Chicago, Chicago, IL, USA, 8Indiana University, Bloomington, IN, USA

Background: Community Network Driven COVID-19 Testing of Vulnerable Populations in the Central US (C3) is a multi-site Rapid Acceleration of Diagnostics Underserved Populations (RADx-UP) study designed to evaluate an intervention combining Social Network Strategy (SNS) with COVID-19 prevention education messages to improve COVID-19 testing and vaccination among criminal legal involved and/or low-income Hispanic community members most impacted by COVID-19.

Methods: C3 enrolled participants through peer referral across 8 study sites in the central United States — Texas, Louisiana, Arkansas, Indiana and Illinois — from April 2021 to December 2022. Participants were randomized 1:1 to the SNS arm, or to the SNS+r-messaging arm which included a staff-led activity to affirm participants’ values and beliefs plus an educational video aiming to correct misinformation about testing and vaccination. Follow-up assessment for COVID-19 testing (primary outcome) and/or vaccination (secondary outcome among unvaccinated participants) was completed 3 weeks after baseline. Bivariate analyses were used to compare participant characteristics across intervention arms. Logistic regression was used to compare primary and secondary outcomes, controlling for site differences.

Results: A total of 1328 participants enrolled, with 661 in the SNS arm and 667 in the SNS+r-messaging arm. Overall, participants identified as 39.8% Black (n=529), 32.4% Hispanic (n=430), and 51.3% (n=681) assigned female at birth. 41.6% (n=553) of participants reported criminal legal involvement prior to enrollment, with no differences between arms by sociodemographics. High numbers of participants were tested (66.3%) and unvaccinated participants vaccinated (11.9%) at three-week follow-up across both arms. Compared to the SNS arm, there were no differences between testing (OR 1.07, 95% CI 0.85, 1.34, p=0.20) or vaccination rates (OR 1.49, 95% CI 0.79, 2.81, p=0.48) among participants in the SNS+r-messaging arm.

Conclusion: Adding educational material including testimonial videos and misinformation correction to the Social Network Strategy (SNS) did not increase testing or vaccination compared to the SNS alone. COVID-19 testing and vaccination rates at follow-up were high among community members in both arms of the SNS intervention. Additional work is needed to address COVID-19 misinformation and implement SNS to increase testing and vaccination rates among communities most impacted by COVID-19 in the United States.

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1096  Targeting Key Populations With HIV Testing Services in Emergency Care in Nairobi, Kenya
Joshua Smith-Sreen1, Beatrice Ngila2, John W. Maina1, Sankei Pirirei2, John Kinuthia2, David Bukusi1, Harriet Waweru3, Rose Bosire1, Daniel Ojuka1, David Katz2, Carey Farquhar3, Michael Mello4, Adam Aluisio5
1Brown University, Providence, RI, USA, 2University of Washington, Seattle, WA, USA, 3Kenyatta National Hospital, Nairobi, Kenya, 4Kenyatta Medical Research Institute, Nairobi, Kenya, 5University of Nairobi, Nairobi, Kenya

Background: Persons seeking emergency injury care have high-risk profiles for HIV. While facility-based HIV Testing Services (HTS) in Kenya are effective, emergency department (ED) delivery is limited, despite the potential of this venue to reach key populations (KPs) and vulnerable populations (VPs). This study assessed impacts of the HIV Enhanced Access Testing in the Emergency Department (HEATED) program in enhancing HTS delivery for high-risk ED patients.

Methods: The HEATED program employed evidence-based behavior-change interventions to promote ED-HTS utilizing resource reorganization and sensitization on HIV care for KPs/VPs. KPs included commercial sex workers, gay men, men who have sex with men, transgender persons, and persons who inject drugs. VPs included persons 18-24 years, interpersonal violence victims, persons with hazardous alcohol use (HAU). Data were collected in pre-implementation (6 March-16 April 2023), post-implementation (1 May-26 June 2023), with a two-week implementation period. Data were obtained from a sample of enrolled patient participants with injuries and via standard HTS records. Chi-squared tests and risk ratios were computed with Wald confidence intervals.

Results: Among 2,578 injury care encounters, 2,303 (89.3%) patients were screened, 605 (26.3%) met inclusion and were enrolled. Overall, 442 (73.1%) participants identified as a KP (7.7%) or VP (89.4%). Commercial sex workers accounted for 68.4% of KPs, and interpersonal violence victims 23.7% of VPs. HTS delivery per 100 patient encounters increased significantly among KP/VP from pre- to post-implementation (RR=12.2; 95% CI: 7.50-19.68). Post-implementation, HTS delivery was significantly greater among participants identifying as a KP/VP than other enrolled participants (p=0.03). Identification of persons living with HIV (PLHIV) increased non-significantly from pre-implementation (n=26) to post-implementation (n=53) (RR=1.15; 0.75-1.76). 73% of these PLHIV were newly diagnosed, and new diagnoses increased non-significantly across phases (RR=1.26; 0.72-2.12), in addition to linkage of new diagnoses to care (RR=1.13; 0.54-2.25).

Conclusion: The HEATED program increased HTS delivery targeting KP/VP and enhanced identification and linkage of new HIV diagnoses, suggesting that broader implementation in Kenya ED settings could support service improvement for high-risk persons already in contact with health systems, who are integral to achieving HIV control targets.
Toward Near-Patient HIV Drug Level Feedback: Implementing an

Method for Analyzing Cabotegravir and Rilpivirine in Hair May Help

Identify Risk Factors for Failure

Background: Long-acting (LA) cabotegravir (CAB) and rilpivirine (RPV) is a promising approach to improve virologic suppression, including among those with adherence challenges. However, resistance can occur among virologic failures. Risk factors for virologic failure defined in the clinical trials include high body mass index (BMI) which can decrease CAB levels, low RPV troughs, HIV subtypes A1/A6 and RPV resistance mutations. Population pharmacokinetic (PK) models of CAB and RPV are sparse given limited real-world roll-out but may help define risk factors for low levels and failure. LA therapies may need long-term metrics to define exposure as exemplified by hair levels. The UCSF Hair Analytical Laboratory (HAL) has experience in validating methods to analyze antiretrovirals in hair.

Methods: For CAB and RPV, the two drugs were extracted from hair from a patient on LA CAB/RPV (approximately 2 mg) into a mixture of methanol and trifluoroacetic acid, which included stable isotopic internal standards. This extraction was carried out through overnight incubation at 37°C. The sample was then evaporated to dryness and reconstituted for analysis via liquid chromatography/mass spectrometry (LC-MS/MS) using an Agilent Infinity II 1290 system coupled with a triple quadrupole mass spectrometer. To separate CAB and RPV, a gradient analysis was performed using a mobile phase composed of methanol, water, formic acid, and acetic acid over 100% methanol. A reverse phase column was used for the separation of CAB and RPV.

Results: Detection of CAB, [13C, 2H2, 15N]-CAB, RPV, and RPV-d6 was achieved using multiple reaction monitoring. Intra-day precision ranged from 1.98% to 4.88% for CAB and 1.31% to 4.09% for RPV, and intra-day accuracy ranged from -9.44% to +10.8% for CAB and -15.0% to +6.92% for RPV. The quantitative linear dynamic standard curve range for CAB was determined to be 0.00625 to 351 ng/mg hair, and for RPV, the range was 0.0625 to 32.0 ng/mg hair.

Conclusion: The UCSF HAL has developed and validated a method to analyze CAB and RPV in hair samples. The UCSF HIV Clinic (Ward 86) has ~250 patients on long-acting CAB/RPV with demographics typically assessed in population PK models (age, sex, BMI, etc.) available in the medical record. A research study to analyze CAB and RPV levels in hair and plasma, construct population PK models, and define risk factors for low LA CAB/RPV exposure, portending virologic failure, will be launched at Ward 86 using the hair method described here. The figure, table, or graphic for this abstract has been removed.

Full Pol-Gene PCR and Rapid ONT Library Preparation for Accurate Drug Resistance Sequencing

Background: Oxford Nanopore Technologies (ONT) third generation sequencing has the benefit of long read-length that allows the efficient and rapid sequencing of long DNA fragments. With the global rollout of integrase strand transfer inhibitors for treatment naïve and treatment experienced patients, drug resistance sequencing increasingly requires the sequencing of the protease (PR), reverse transcriptase (RT) and integrase (IN). We have therefore developed a rapid assay workflow that involves PCR amplification of a ~3 kb target, including all drug resistance mutations in pol, with rapid ONT barcode ligation followed by ONT real-time sequencing.

Methods: HIV RNA was extracted with the NucliSens EasyMag (bioMerieux) and amplified with a one-step reverse transcriptase PCR and nested PCR amplifying the ~3 kb PR-RT-IN target. Library preparation was with a fast ONT barcoding kit. We used residual HIV viral load samples (HIVVL) (n=71) to assess amplification success at various viral load strata. Residual samples from patients that had undergone Sanger Sequencing for protease and reverse transcriptase (n=35) with Applied Biosystems™ HIV-1 Genotyping Kit (ThermoFisher Scientific) and a published homebrew assay for integrase (Van Laethem 2008)
1101 User-Friendly and Efficient Multiplex HIV, Syphilis, and Hepatitis B & C Self-Screening in Thailand

Nicolás Salvadori1, Jullapong Achalapong1, Titipan Akarasereenont1, Sawitree Nangola2, Chiraphat Klyoppan3, Eakkapote Prompunt4, Naruepon Wongpluesin5, Surachet Arumonthong6, Wootichai Khumduang7, Nicole Ngo-Yiang-Huong8, Sakom Pomprasert9, Sunet Omwangwande10, Gonzague Jourdain11, for the Napneung Project Team
1Chiang Mai University, Chiang Mai, Thailand, 2Chiang Rai Prachanukroh Hospital, Chiang Rai, Thailand, 3IDS Clinic of the Office of Disease Prevention and Control Region 1, Chiang Mai, Thailand, 4University of Phayao, Phayao, Thailand, 5Mae Lao Hospital, Mae Lao, Thailand, 6Ministry of Public Health, Nonthaburi, Thailand, 7Institut de Recherche pour le Développement, Chiang Mai, Thailand

Background: Affordable and reliable rapid test kits pave the way for widespread screening. However, it is crucial that the process be user-friendly, quick, confidential, cost-effective and include medical support when needed. It should also save healthcare workers (HCW) time. The Napneung project is working towards efficient HIV screening methods.

Methods: The Napneung project offers free and anonymous self-screening for HIV, syphilis and hepatitis B and C at five locations in northern Thailand to anyone aged ≥15 years. The medical team in collaboration with IT specialists developed an advanced web app, available in several languages, to automate most processes: online appointments to avoid queues and waiting times, instructions for user-guided self-screening, and standardized information provided on sexually transmitted infections while awaiting results. The app allows a HCW to assist multiple users at once. Only two drops of blood from a finger prick are needed to screen for the four infections. If a test is positive, confirmatory tests are performed, then post-test counseling and personalized referral for evaluation and treatment are provided. High-risk HIV-negative users are encouraged to start PrEP and re-test regularly. For demand creation, the service is promoted online through social media and search engines, and offline through posters and vouchers. No incentives are offered.

Results: 16,733 screening sessions were provided to 12,175 users between Oct 19, 2015 and Jun 7, 2023. 49% of users were male at birth, 45% were aged 15–24 years, 17% reported being MSM or transgender women, and 63% had never tested for HIV. Median turnaround time from arrival of test results was 36 (30–44) minutes. >99% reported being satisfied with the self-screening process. 222 (1.7%, excluding those already aware) were newly diagnosed with HIV (50% had never tested for HIV, and 62% were MSM or transgender women), 230 (1.9%) with syphilis, 193 (1.6%) with hepatitis B and 67 (0.5%) with hepatitis C. The relatively high median CD4 count at diagnosis (370 cells/mm³, versus 200 nationwide) and recency assay testing showed that the service is used shortly after HIV acquisition. 95% of users newly diagnosed with HIV subsequently confirmed that they initiated treatment.

Conclusion: This effective, well-received and affordable system saves time for HCW and users. Associated multiplex tests with IT resources enables the integration of efforts to fight these four chronic infections without additional burden.

1102 Effectiveness of Using HIV Self-Tests as an Incentive for Testing Within Assisted Partner Services

Unmessa Roy Paladi1, David Katz2, George Dieno3, James P. Hughes1, Harsha Thirumurthy1, Harison Lagat4, Sarah Masyuko4, Monisha Sharma5, Paul Macharia6, Rose Bosire7, Mary Mugambi8, Edward Karithi9, Carey Farquhar10
1University of Washington, Seattle, WA, USA, 2PATH, Kisumu, Kenya, 3University of Pennsylvania, Philadelphia, PA, USA, 4Pathetta National Hospital, Nairobi, Kenya, 5Kenya Medical Research Institute, Nairobi, Kenya, 6Ministry of Health, Nairobi, Kenya, 7PATH, Nairobi, Kenya

Background: Assisted Partner Services (APS) are used shortly after HIV acquisition. 95% of users newly diagnosed with HIV were recommended to offer HIV self-testing (HIVST) within APS in Kenya. However, the process is user-friendly, quick, confidential, cost-effective and include medical support when needed. It should also save healthcare workers (HCW) time. The Napneung project is working towards efficient HIV screening methods.

Methods: The Napneung project offers free and anonymous self-screening for HIV, syphilis and hepatitis B and C at five locations in northern Thailand to anyone aged ≥15 years. The medical team in collaboration with IT specialists developed an advanced web app, available in several languages, to automate most processes: online appointments to avoid queues and waiting times, instructions for user-guided self-screening, and standardized information provided on sexually transmitted infections while awaiting results. The app allows a HCW to assist multiple users at once. Only two drops of blood from a finger prick are needed to screen for the four infections. If a test is positive, confirmatory tests are performed, then post-test counseling and personalized referral for evaluation and treatment are provided. High-risk HIV-negative users are encouraged to start PrEP and re-test regularly. For demand creation, the service is promoted online through social media and search engines, and offline through posters and vouchers. No incentives are offered.

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Conclusion: This effective, well-received and affordable system saves time for HCW and users. Associated multiplex tests with IT resources enables the integration of efforts to fight these four chronic infections without additional burden.

1101 A Rapid Emtricitabine Urine Lateral Flow Assay for Monitoring Daily Adherence to ART and PrEP

Thomas H. Vanderford1, Xierong Wei2, A. S. Youngpairoj3, Ayanna Green4, Richard Haaland5, Jeffrey A. Johnson6, Waldid Heneine7
1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Centers for Disease Control and Prevention, Gabonone, Botswana

Background: Adherence monitoring at point-of-care is challenging in the absence of rapid and accurate measures of antiretroviral drug levels. Emtricitabine (FTC) is a fixed dose component of most antiretroviral therapy (ART) and pre-exposure prophylaxis (PrEP) regimens that is rapidly excreted in urine at high concentrations making it a suitable candidate for adherence measurement. We developed a test for daily adherence to FTC with a low-cost, rapid lateral flow assay (LFA) that detects FTC in urine. Here, we evaluate the performance of this LFA to discriminate daily adherence in people with HIV (PWH) on ART and individuals on daily PrEP.

Methods: FTC-specific LFAs were designed in collaboration with a commercial manufacturer using a monoclonal antibody developed by our group. FTC LFA measurements were objectively read by a CubeReader (Chembio, Germany) with urine FTC concentrations >20 µg/mL indicating adherence to daily dosing. LFA performance (sensitivity and specificity) was evaluated on 273 urine samples which included (a) drug naïve, uninfected individuals (n = 62), (b) PWH prescribed ART (n = 67), and (c) people on PrEP (n = 144). FTC concentrations in urine and in plasma were measured by liquid chromatography tandem mass spectrometry for comparison with LFA results and to confirm adherence, respectively.

Results: The FTC LFA provides results within 20 minutes. We evaluated the LFA for its ability to discriminate adherent from non-adherent individuals. FTC levels in plasma were used to stratify individuals for adherence based on a cutoff for daily adherence (>49 ng/mL plasma FTC concentration) optimized for greater than 90% sensitivity and specificity (Hendrix et al, 2016). The sensitivity of the LFA was 87.3% and its specificity was 93.5%. Thus, the FTC LFA performed well with >90% sensitivity and specificity (Hendrix et al, 2016). The sensitivity of the daily adherence (ptide 49 ng/mL plasma FTC concentration) optimized for greater in plasma were used to stratify individuals for adherence based on a cutoff for

Conclusion: The novel fast ONT-based full pol-gene provides an alternative to Sanger Sequencing, especially in patients with higher viral loads. Discordant cases with Sanger Sequencing are likely a combination of PCR resampling error and small differences in the sequencing platforms. Our assay combined with NanoRecall interpretation provides an assay suitable for the use case of drug resistance surveillance and individual patient management in resource limited settings.

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Methods: FTC-specific LFAs were designed in collaboration with a commercial manufacturer using a monoclonal antibody developed by our group. FTC LFA measurements were objectively read by a CubeReader (Chembio, Germany) with urine FTC concentrations >20 µg/mL indicating adherence to daily dosing. LFA performance (sensitivity and specificity) was evaluated on 273 urine samples which included (a) drug naïve, uninfected individuals (n = 62), (b) PWH prescribed ART (n = 67), and (c) people on PrEP (n = 144). FTC concentrations in urine and in plasma were measured by liquid chromatography tandem mass spectrometry for comparison with LFA results and to confirm adherence, respectively.

Results: The FTC LFA provides results within 20 minutes. We evaluated the LFA for its ability to discriminate adherent from non-adherent individuals. FTC levels in plasma were used to stratify individuals for adherence based on a cutoff for daily adherence (>49 ng/mL plasma FTC concentration) optimized for greater than 90% sensitivity and specificity (Hendrix et al, 2016). The sensitivity of the LFA was 87.3% and its specificity was 93.5%. Thus, the FTC LFA performed well with >90% sensitivity and specificity (Hendrix et al, 2016). The sensitivity of the daily adherence (ptide 49 ng/mL plasma FTC concentration) optimized for greater in plasma were used to stratify individuals for adherence based on a cutoff for

Conclusion: The novel fast ONT-based full pol-gene provides an alternative to Sanger Sequencing, especially in patients with higher viral loads. Discordant cases with Sanger Sequencing are likely a combination of PCR resampling error and small differences in the sequencing platforms. Our assay combined with NanoRecall interpretation provides an assay suitable for the use case of drug resistance surveillance and individual patient management in resource limited settings.
estimate the effect of the incentive of two HIVSTs on overall and first-time testing among APS partners who reported no prior HIV diagnosis.

**Results:** From March 2021–June 2022, 1127 index clients received APS and named 2155 partners, of whom 2333 reported a prior HIV diagnosis and were excluded from analyses, resulting in 5822 remaining partners: pre-implementation, 1489 (40.8%) and 2157 (59.2%) partners were in the control and intervention arms, and post-implementation, 815 (37.5%) and 1361 (62.5%) partners were in the control and intervention arms. Pre-implementation, 1422/1489 (95.5%) in the control arm tested for HIV versus 2111/2157 (97.9%) in the intervention arm; post-implementation the numbers were 699/815 (85.8%) and 1204/1361 (88.5%) in the control and intervention arms. Comparing partners offered one vs. two HIVSTs showed no difference in HIV testing (DID relative risk [RR]: 1.01, 95%Confidence Interval [CI]: 0.951-1.07) or first-time testing (DID RR: 1.23, 95%CI: 0.671-2.24). Of partners offered a second HIVST, 940/1204 (78.1%) opted for HIVST, 322/940 (34.3%) picked up two kits, and 231/322 (71.7%) reported that the second kit encouraged HIV testing.

**Conclusion:** Offering a second HIVST to partners of index clients as an incentive within APS did not significantly impact HIV testing or first-time testing, likely since HIV testing rates were already high at baseline.

**1103 Increased HIV RNA Testing of PrEP Users Following 2021 CDC Guidance:**

Weiming Zhu, Ya-Lin A. Huang, Kevin Delaney, Athena Kourts, Karen W. Hoover

Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** Persons using PrEP cannot have ambiguous HIV test results for viral inhibition during use that might delay the diagnosis of HIV infection. In December 2021, CDC recommended HIV RNA nucleic acid testing (NAT) in addition to HIV antigen/antibody (Ag/Ab) testing at initiation of long-acting cabotegravir injections (CAB-LA), and for follow-up testing of users of all PrEP regimens. We estimated the impact of CDC guidance on combined HIV RNA-Ag/Ab testing associated with PrEP use in the U.S.

**Methods:** We analyzed data in the HealthVerity database of longitudinally labeled prescriptions and HIV testing laboratory data from 2019 through June 2023. We identified persons prescribed oral or injectable PrEP and extracted their HIV testing records. A combined test is defined as Ag/Ab and RNA tests ordered within an interval of less than 7 days. We defined testing at PrEP initiation if it occurred ≥7 days from the first prescription, and testing for follow-up if it occurred from 30 days after the first prescription to 14 days after the end of the last prescription. For oral PrEP users, we estimated the rate of combined testing among patients’ follow-up testing pre- and post-2021 CDC guidelines. For CAB-LA users, we estimated the rate at PrEP initiation or follow-up in the post-2021 guideline period.

**Results:** We identified 10,856 oral PrEP users with follow-up testing during the pre-2021 guideline period; 552 (5%) received combined testing, 613 (6%) had NAT only, and 9,592 (88%) had Ag/Ab testing only. During the post- updated guideline period, among 10,972 oral PrEP users with follow-up tests, 3,109 (28%) received combined testing, 1,066 (10%) had NAT only, and 6,891 (61%) had Ag/Ab only. Among 691 CAB-LA users only; 130 (19%) received combined testing at PrEP initiation. Among 540 CAB-LA users with follow-up tests, 333 (62%) received combined testing, 118 (22%) had NAT only, and 78 (14%) had Ag/Ab testing only.

**Conclusion:** The rate of combined HIV RNA-Ag/Ab testing among oral PrEP users increased substantially after CDC published updated guidance in 2021 but the rates are sub-optimal, as is the rate of combined testing at CAB-LA initiation. However, the rate of combined testing during CAB-LA follow-up is higher. Further research is required to investigate the extent of long-acting viral inhibition syndrome in individuals using PrEP, as well as to assess the cost-effectiveness of HIV RNA testing for PrEP users of different regimens.

**1104 Evidence of HIV Infection Prior to Rapid Antibody Test Positivity in PrEP Breakthrough Infections**

Vivian I. Avelino-Silva,1 Mars Stone,1 Sonia Bakkour2,1 Clara Di Germinario,2 Michael Schmidt,2 Ashtyn Conway,4 David Wright,3 Brian Custer1, Steven Kleiman1, Jairam Lingappa1, Patricia Defechereux1, Megha Mehrotra3, Robert Grant1, Michael P. Busch5, Philip P. Norris6

1Weslaw Research Institute, San Francisco, CA, USA, 2German Red Cross, Frankfurt, Germany, 3Bloodworks Northwest Research Institute, Seattle, WA, USA, 4Westcot, Inc. Rockville, MD, USA, 5University of British Columbia, Vancouver, Canada, 6University of Washington, Seattle, WA, USA

**Background:** Exposure to antiretrovirals in PrEP users with breakthrough infection can suppress viral replication early after HIV acquisition, leading to delayed or inconsistent seroconversion. Limitations in HIV diagnostic accuracy in this context are higher for assays with lower sensitivity, such as point-of-care rapid antibody tests (RT).

**Methods:** We obtained plasma samples from visits prior to the first positive RT from 251 participants with incident HIV during randomized PrEP trials (IPEx and PrEP): which used 3rd generation RT (state-of-the-art for HIV diagnosis at the time). Samples were categorized into time intervals and analyzed using higher performance tests (Abbott Alinity HIV Ag/Ab Combo, Roche Elecsys HIV Duo, and Ortho VITROS HIV Combo for antigen/antibodies detection; Roche cobas MPX for RNA detection) to address positivity comparing participants assigned to active PrEP (N=101) to those assigned to placebo (N=150).

**Results:** A large percentage of samples had positive results in higher performance tests before infection detection by RT. Rates were consistently higher for participants assigned to active PrEP compared to placebo, with statistical significance for the <8 weeks timeframe in 2 of 4 assays. The combination of RNA and antigen/antibody tests further increased overall positivity, although not reaching statistical significance when compared to antigen/antibodies tests alone. We also analyzed signal-to-cutoff index results in antigen/antibodies tests over time prior to the first positive RT, by treatment assignment; levels were somewhat higher, and positivity was identified earlier in participants assigned to active PrEP, suggesting longer duration of infection prior to the first positive RT.

**Conclusion:** Compared to point-of-care RT, higher sensitivity lab-based serologic and nucleic acid detection assays can enhance the identification of breakthrough HIV infections in PrEP users. Our findings suggest consideration of adopting higher sensitivity tests in PrEP programs that rely exclusively on point-of-care 3rd generation RT for follow-up. For blood banks, our results reinforce that screening with parallel lab-based serologic and RNA tests should be ensured. Study limitations include sample storage beyond the manufacturers’ labeled claims and possible laboratory errors.

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**1105 Costs and Clinic Flow of Point-of-Care Urine Tenofenovir and HIV Viral Load Testing in South Africa**

Melody Wang1, Pravi Moodley1, Meliungisi Khamile, Elliot Bulo, Makkosazane Zondi, Keshani Naidoo,1 Yukteshwar Sookrah,2 Jienchi Dorward,3 Nigel Garrett4, Paul K. Drain1, Manisha Sharma1

1University of Washington, Seattle, WA, USA, 2National Health Laboratory Service (NHLS), Durban, South Africa, 3Centre for the AIDS Program of Research in South Africa (CAPRISA), Durban, South Africa, 4Ethekwini Municipality Health Unit, Durban, South Africa, 5University of KwaZulu-Natal, Durban, South Africa, 6University of Oxford, Oxford, United Kingdom

**Background:** Point-of-care (POC) urine tenofovir (TFV) tests can monitor real-time ART adherence in clinics and complement POC viral load (VL) tests for HIV treatment management. However, clinic flow and implementation cost of both tests is uncertain. We sought to estimate costs of integrated POC TFV and VL testing for persons initiating ART in South Africa to inform implementation of new diagnostic technologies for person-centered HIV care.

**Methods:** We conducted microcosting within STREAM HIV, a randomized implementation trial evaluating POC HIV TFV and VL testing in a government clinic in KwaZulu-Natal, South Africa. We collected time- and motion data to assess staff and client time needed for POC TFV testing. We estimated financial and economic costs for capital, clinic consumables, and personnel using a provider perspective. We updated costs for POC VL testing from 2018 to 2022 USD using the gross domestic product price deflator and new prices for test cartridges. We estimated instrument costs assuming five-year lifespans with a 3% annual discount rate.

**Results:** The per-client test costs, including both financial and economic costs, of POC TFV and VL testing were USD $12 and $21, respectively, assuming a clinic
volume of individuals 50 initiating ART clients per month. Key cost drivers for POC TFV and VL tests were the test strip and cartridge consumables, accounting for 52% and 70% of total test costs, respectively. The median time clients spent in the clinic for a visit with a POC TFV test was 49:31 (minutes: seconds). The POC TFV testing took a median time of 9:36 (19% of total clinic visit) including sample collection, sample loading, TFV test processing, and counselling provision based on test results. Overall, 29% of the clinic visit time included direct clinical care and assessment with a provider, with clients spending a median time of 14:15 getting vital checked, adherence monitored via POC TFV testing, and collecting ART. Waiting in line for ART took most (48%) of the clinic visit time with a median wait time of 23:42.

Conclusion: POC TFV testing is low-cost, requires less than 10 minutes, and may be feasible to implement in South African clinics. Findings can inform the policy decisions and budgetary planning for ART monitoring, in South Africa and similar settings that are considering using POC TFV and VL testing to support adherence monitoring in lieu of lab-based VL testing.

1106 A Capillary Blood-Based Self-Collection Method for Monitoring HIV Viral Load During ART Interruption
Livio Azzeni,1, 2, 3 Daniel S. Rosenbloom,1, 2 Jessica Marie Morris,4 Jennifer Nguyen,4 Brian N. Ross,2 Matthew Fair, Emmanouil Papasavvas,5 Kenneth Lynn, Emily Hiscott, Brian Squadroni,6 Brad R. Evans,7 Pablo Tebas,8 Karam Mounzer9, Bonnie Howell,1 Luis J. Montaner1

1 Wistar Institute, Philadelphia, PA, USA, 2 Merck Research Laboratories, Rahway, NJ, USA, 3 Merck & Co, Inc, Palo Alto, CA, USA, 4 University of Pennsylvania, Philadelphia, PA, USA, 5 Philadelphia FIGHT, Philadelphia, PA, USA, 6 Merck & Co, Inc, Upper Gwynedd, PA, USA, 7 Merck & Co, Inc, Rahway, NJ, USA

Background: Multiple barriers limit participation of people with HIV (PWH) in studies in which frequent viral load (VL) monitoring is required such as cure studies involving a treatment interruption (ATI). Convenient, home-based VL testing may increase equitable participation. Here we report a novel VL test based on capillary blood collection, comparing its specificity and sensitivity to conventional clinic-based plasma-based VL (pVL).

Methods: We enrolled 21 PWH (5% female, 67% African American, mean age of 51 years) undergoing planned ATIs as part of the BEAT-HIV trial (NCT03588715). At contiguous visits (mean 20.5), we collected A) capillary blood using two 4-well Tasso-M50 devices, and B) matched plasma samples. Between visits, participants self-collected capillary blood using two devices that were mailed back (home collection). We employed a robotic automation process for magnetic bead RNA extraction and RT-qPCR readout with dual LTR/GAG FAM-labeled primers that amplify on the same fluorescence channel. Samples and standards underwent qPCR in duplicate.

Results: The devices were well-accepted, with a collection failure rate <10%. We analyzed a total of 5392 M50 PCR reads (3058 clinic, 2334 home collection). Among the 14 participants where M50 background levels could be determined from ≥ 4 weeks of suppressive treatment, M50 VL 2x above median background was predictive of matched pVL ≥ 200 c/mL (Figure 1. Sensitivity 66%, specificity 81%, PPV 71%, NPV 76%, ROC curve AUC 85%). When M50 VL was < median background, pVL was reliably low (NPV 84% for pVL ≥ 200 c/mL, NPV 90% for pVL ≥ 1000 c/mL). 13 of 14 (93%) participants experienced an increase in M50 VL at the collection immediately following pVL rebound ≥ 200 c/mL (median increase 9-fold, range 1.8 - to >1000-fold); the remaining participant experienced an increased M50 VL at the subsequent collection (to 1.6-fold above background). An M50 VL ≥ 2x background was always followed by pVL rebound ≥ 200 c/mL within 4 weeks.

Conclusion: Our new M50 assay showed good NPV for pVL > 200 c/mL in a cohort of PWH undergoing ATI. This suggests that, upon clinical validation, the assay could be used for home monitoring of VL during ATIs. This approach could enhance equitable participation in HIV research by minimizing participants’ visit burden. Other potential applications, such as monitoring new infections in prophylaxis studies or assessing changes in residual cell-associated HIV in cure studies, are warranted.

1107 First Case of HIV Seroconversion With Emergence of InSTI Resistance on CAB-LA PreP in Routine Care

Catherine A. Koss1, Monica Gandhi2, Elias K. Halvass3, Hideaki Okochi,4 Carolyn Chu,5 David V. Gildener,6 Lisa Georgetti Gomez, Amy L. Heaps7, Amy A. Conroy8, Michael Tran9, Bhavna Chohan10, James Ayekoo11, John W. Mellors12, Urvi M. Parikh13, for the SeroPreP Study Team

1 University of California San Francisco, San Francisco, CA, USA, 2 University of Pittsburgh, Pittsburgh, PA, USA, 3 Men’s Health Foundation, Los Angeles, CA, USA, 4 University of Washington, Seattle, WA, USA, 5 Kenya Medical Research Institute, Nairobi, Kenya

Background: Long-acting cabotegravir (CAB-LA) PrEP is highly effective but delayed diagnoses and INSTI resistance were observed with incident infections in registration trials. As CAB is scaled up, continued vigilance is needed to assess HIV acquisition, pharmacokinetics (PK), resistance, and ART outcomes after infections. We report the first case outside of trials, to our knowledge, of HIV infection on CAB with emergence of INSTI resistance.

Methods: A 23-year-old gender-nonbinary person, male at birth (on estradiol, spironolactone; BMI 19) with history of CAB PreP use restarted CAB 6 months after discontinuation (Figure). On Day(D) 20 before CAB restart, HIV Ag/Ab was non-reactive (NR) and HIV RNA not detected (ND). Point-of-care HIV Ab was NR on the day of the 1st CAB injection (D0). On D28, when the 2nd CAB injection was given, the HIV Ag/Ab was reactive (index value 1.24), HIV RNA 451 c/mL. The patient enrolled in SeroPreP, a study of breakthrough infections on PrEP across the U.S. Blood and hair samples were collected for sensitive diagnostic, PK, and resistance assays. RNA below routine thresholds was quantified via single copy assay (SCA). INSTI mutations were identified by single genome sequencing (SGS) of full-length integrase, and compared to the partner’s viral genotype (GenoSure PrIME NGS). We measured CAB levels by LC-MS/MS in plasma and segmental hair analysis.

Results: SGS of plasma HIV-1 RNA identified INSTI mutations Q148R in 2/24 and A128T in 1/24 sequences 2 days after diagnosis and the 2nd CAB injection (D30), while the commercial genotype assay (HIV RNA 410 c/mL). The partner’s plasma viral genotype had no INSTI mutations, indicating CAB resistant variants arose after HIV infection and were selected by CAB in this case. Plasma from D42 (14 days post-diagnosis/8 days post-ART start [DRV/c/TAF]) was HIV-1 Ab reactive/ HIF-1 Ag NR (BioPlex 2200), HIV RNA 23 c/mL (SCA). INSTI level 33.7 pg/mL CAB levels in hair were 0.19 ng/mg/3 weeks pre-diagnosis and 0.95 ng/mg/3 weeks post-diagnosis and 2nd injection. HIV RNA was ND 1 month post-ART start.

Conclusion: In this first case in routine care of HIV infection on CAB with emergence of INSTI resistance, infection likely occurred around the time of CAB reintiation. CAB resistance emerged rapidly and was only detected by a sensitive research assay. This case highlights the value of RNA testing as close as possible to CAB start and the need to assess resistance, PK, and treatment outcomes to inform clinical and public health strategies.

1108 Case of Seroconversion on CAB-LA: Timeline of ART Administration and Research and Clinical Testing

A diagram showing the timeline of ART administration and research and clinical testing.
1109 Testing Cabotegravir for HIV Preexposure Prophylaxis in a Large Urban HIV Clinic

Christian Turner1, Gabriel Wagner1, Allan Pfeifer2, Aaron Wilcott2, Tyler Lonergan3, Lucas Hill4, Jill Blumenthal5
1University of California San Diego, La Jolla, CA, USA, 2University of California San Diego, San Diego, CA, USA

Background: Long-acting cabotegravir (CAB-LA) offers a novel HIV PrEP option for individuals unable to effectively take or tolerate oral PrEP and may expand access to PrEP to populations that have been difficult to reach. We describe the development of a CAB-LA program in a large urban HIV clinic and characterize the program’s first patient cohort.

Methods: The UC San Diego Owen Clinic is a Ryan White-funded HIV primary care clinic that also provides PrEP to over 400 patients. Starting in January 2021, individuals interested in initiating CAB-LA were referred to a PrEP pharmacist and a navigator for an insurance check and as soon-as-same-day initiation with on-site delivery of injection. Patients were included who initiated CAB-LA and had at least one follow-up injection. Demographic data were collected through EMR review, and reasons for discontinuation and missed doses were documented and continually reviewed. Logistic regression was performed to evaluate predictors of discontinuing CAB-LA.

Results: Between 1/2021 and 6/2022, providers referred 215 patients to the CAB-LA program, and 162 patients (75%) received at least one injection. Median age was 31.5 (IQR 27.39, among 48% White, 3% Black and 30% other race individuals with 40% (n=65) reporting Hispanic ethnicity. Ninety-one percent (n=148) identified as cisgender male, and 78% (n=126) reported previous oral PrEP use. Twenty-six (16%) discontinued therapy, with n=11 citing injection site reactions or pain caused by injection. Other reasons for discontinuation included moving or transferring care (n=4), change in HIV risk (n=3), insurance change (n=3), lost-to-followup (n=3), and other side effects (n=2). Ninety patients were covered through pharmacy benefits (42 with Medicaid, 48 with private insurance), 46 through medical benefits, and 28 by a patient assistance program (PAP). Younger age (OR 1.05, 95% CI 0.99, 1.09, p=0.08), having non-PAP coverage (OR 12.5, 95% CI 1.4-111.11, p=0.013), and missing an injection (OR 7.6, 95% CI 1.5-338.65, p=0.023) were associated with discontinuation of CAB-LA.

Conclusion: We observed robust uptake of CAB-LA for HIV prevention among individuals with a clinical need or preference for non-oral PrEP. Most were prior oral PrEP users, highlighting the desire for choice among people who use PrEP for HIV prevention. Younger individuals, those without patient assistance program support and those who miss injection doses may need additional support to keep them engaged in CAB-LA.

1110 Preexposure Prophylaxis With Cabotegravir Long-Acting Injectable in the OPERA Cohort

Anthony Mills1, Laurence Brunet1, Karam Mounzer1, Michael B. Wohlfeiler2, Kevin R. Frost3, Ricky K. Hud4, Gerald Pierone1, Michael Sension5, Michael Aboud5, Philip C. Lackey2, Tyler Lonergan5, Michael B. Wohlfeiler1, Andrew J. Frick3, Carolyn Brown3, Gayathri Sridhar4, Leigh Ragone3, Jean Van Wyk5, Anthony Mills1, Steven Santiago5, Richard A. Elion5, Vani Vannappagari1, Epividian, Raleigh, NC, USA, 2Wake Forest University, Winston-Salem, NC, USA, 3Trio Health, Inc, Louisville, CO, USA, 4ViiV Healthcare, Durham, NC, USA, 5Trio Community Health, Inc, Miami, FL, USA

Background: Cabotegravir (CAB) long acting (LA) for pre-exposure prophylaxis (PrEP) was approved in the United States in December 2021 to reduce the risk of sexually acquired HIV-1 infection. The Centers for Disease Control and Prevention (CDC) guidelines state that both HIV antigen (Ag)/antibody (Ab) and HIV RNA testing should be conducted at every injection. Real-world testing, effectiveness, and adherence were assessed among individuals initiating CAB LA for PrEP in the US.

Methods: Individuals without HIV initiating CAB LA for PrEP were identified from electronic health record data in the Trio Health cohort between December 2021-May 2023. HIV testing and incidence were assessed among individuals with at least one injection of CAB LA. HIV testing was assessed at baseline within 90 days prior to the first injection and during follow-up within ±14 days of injection. Incident HIV was identified with either a positive HIV Ag/Ab lab result with confirmatory HIV RNA test or one detectable HIV RNA. Adherence was assessed among individuals with ≥2 injections of CAB LA. On-time injections were defined as occurring within ±7 days of target date and missed injections were defined as a missed injection cycle.

Results: Of 560 CAB LA for PrEP users identified, 13% were women, 32% Black, 29% Hispanic, and 26% had a BMI ≥30; median age was 31 years (IQR 25-38). Within 12 months prior to CAB LA for PrEP initiation, 42% of individuals had an STI diagnosed and the median number of HIV tests was 3 (IQR, 2-5). The initiation injections were completed by 498 individuals (89%) who had a median of 4 injections over a median 7 months of follow-up. Of the 498 with ≥2 injections, 7% discontinued CAB LA for PrEP (Table). Over two-thirds of CAB LA users received all injections on-time and 11% missed an injection (Table). Those with prior PrEP use were more likely to have delayed or missed injections (32%) than those without (21%), with a non-statistically significant odds ratio of 1.78 (95% CI: 0.91, 3.47). Oral bridging was not well documented in EHR; oral PrEP for oral bridging might be prescribed at the start of CAB LA injections but actual use is difficult to ascertain. There was 1 HIV seroconversion concurrent with third injection (all on time); HIV testing at oral PrEP start but not at CAB LA PrEP start.

Conclusion: Early adoption of CAB LA for PrEP was successful in OPERA, a US cohort of EHR from routine clinical care. A sizeable proportion of CAB LA PrEP initiators had diagnoses of STI and multiple HIV tests in the preceding 12 months. While 11% of individuals missed an injection, this may be an overestimate of true therapeutic gaps if oral bridging was used.

Table. Follow-up and injection patterns among individuals who completed initiation of CAB LA for PrEP

<table>
<thead>
<tr>
<th>Complication Initiation</th>
<th>n=646</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months of Follow-up, median (IQR)</td>
<td>7.0 (4.7, 8.5)</td>
</tr>
<tr>
<td>Number of injections received, median (IQR)</td>
<td>4.9 (3.8)</td>
</tr>
<tr>
<td>Discontinuation (≥ 128 days without injection), n (%)</td>
<td>34 (5)</td>
</tr>
<tr>
<td>Any delayed injection (≥ 14 days after target date), n (%)</td>
<td>97 (15)</td>
</tr>
<tr>
<td>Any missed injection (≥ 14 days after target date), n (%)</td>
<td>90 (14)</td>
</tr>
<tr>
<td>Any delayed and/or missed injection, n (%)</td>
<td>154 (24)</td>
</tr>
</tbody>
</table>

*Complete initiation: init. 2 injections received within 7 days apart
the early days of CAB LA for PrEP did not align with the CDC testing guidelines among a significant proportion of users.

### Drug Level Monitoring for PrEP Users: Feasible and Acceptable, but Is It Necessary?

**Erica R. Pool,** Abigail Severn, Jose L. Paredes Sosa, Oliver Stirrup, Claire-Marie Mullender, Marzia Fiorino, Manik Kohli, Irfaan Maan, Rhiannon Owen, Deirdre Sally, Emmi Sunnaphera, David Dunn, Richard Gilson, John Saunders University–University of California San Francisco Research Collaboration, Kampala, Uganda, University School of Medicine, Durham, NC, USA, Columbia University, New York, NY, USA, Malawi-Liverpool-Wellcome Trust Centre, Blantyre, Malawi, University of Washington, Seattle, WA, USA, Infectious Disease Institute, Kampala, Uganda, Duke University School of Medicine, Durham, NC, USA, Columbia University, New York, NY, USA, Makerere University–University of California San Francisco Research Collaboration, Kampala, Uganda, University of Alabama at Birmingham, Birmingham, AL, USA

**Background:** Good adherence is vital for PrEP efficacy. Most adherence data come from clinical trials but real-world data for long-term PrEP use are lacking. Optimal support for PrEP adherence is not fully established and tenofovir (TFV) drug level monitoring (DLM) could identify those not taking PrEP. It is not known whether DLM would be acceptable to PrEP users or feasible within clinic.

**Aims:** 1. Is PrEP DLM testing acceptable and feasible? 2. Describe adherence in those attending routine care. 3. Compare biochemically verified to reported adherence.

**Methods:** PrEP users aged >16 years attending a large London sexual health clinic were invited to complete a survey on PrEP use and offered a point of care urine test (UrSure®) for TFV. This detects TFV concentration >1000 ng/mL, consistent with PrEP use in the last 48 hours, validated for emtricitabine-tenofovir disoproxil fumarate (FTDF) and emtricitabine-tenofovir alafenamide fumarate (F/TA).

**Results:** We enrolled 213 people from September 2021–October 2022, 199 completed the survey and DLM test, 14 completed the survey alone. Characteristics of participants were: 94.8% cisgender male; 3.8% trans/nonbinary; 70.9% white; 94.8% gay/bisexual; median age 39 years (IQR 30–51). In the last 12 months 37.6% used any recreational drugs and 10.3% used crystal. PrEP use: 71.8% daily, 23.5% event based dosing (EBD); 88.3% F/TDF and 11.7% F/TAF. Median duration of PrEP use was 4 years, (IQR 2-5, range 1-10). Urine DLM was highly acceptable; 79.8% reported that they were quite/very likely to accept it; when offered, 94.3% completed a DLM test. DLM was feasible; median test completion time was 11.4 minutes (IQR 11.2–12.5), only 2 invalid results. Reported PrEP adherence matched DLM verified adherence in >95% of cases. Almost all participants had PrEP adherence consistent with efficacy; for 96.7% of daily users adherence was consistent with >4 pills/week. For EBD, 73.1% reported >80% of recent condomless sex was covered by PrEP; EBD was often not used with primary partners or partners who are undetectable. Common reasons for missed PrEP were forgetting, running out, busy or not having sex.

**Conclusion:** Point of care PrEP urine DLM was highly acceptable and feasible but in this study did not add to self-reported adherence. PrEP adherence in this population attending for routine care was extremely high despite long-term PrEP use.

### PrEP Counseling Based on a Tenofovir Urine Assay Decreases Overall Non-Adherence Among Kenyan Women

**Monica Gandhi,** David V. Glidden, Matthew A. Spinelli, Charlene Biwoot, Gakuu Maima, Irene Njeno, Catherine Kiptinness, Philix Okello, Pumba Chatterjee, Guohong Wang, Vallery Ogleti, Hidayek Okochi, Deepakha Chakravarty, Neilly R. Muco, Kenneth Nguere

1 University of California San Francisco, San Francisco, CA, USA, 2 Kenya Medical Research Institute, Kilifi, Kenya, 3 Kenya Medical Research Institute, Nairobi, Kenya, 4 Abbott Labs, Abbott Park, IL, USA

**Background:** Adherence to oral pre-exposure prophylaxis (PrEP) with tenofovir (TFV)-based PrEP among women in Africa in non-serodiscordant partnerships can be low based on clinical trial and real-world data. A point-of-care (POC) urine assay to objectively assess TFV in urine was recently developed by our group and POC urine monitoring of adherence-informed counseling (PUMA) increases virologic suppression rates among people with HIV on antiretroviral therapy. We performed a pilot trial examining the impact of PUMA on long-term PrEP adherence among HIV-negative women in Kenya.

**Methods:** A pilot study randomized 100 women in non-serodiscordant partnerships in Thika, Kenya to standard-of-care (SOC) adherence counseling every 3 months for 12 months versus PUMA with visualization of the POC test by providers and participants. Urine tests were collected for 12-month analysis in the SOC arm, without providing results to participants or staff. Hair samples were collected at month 12 to assess the primary outcome of TFV levels in hair as a long-term metric of adherence in both arms. TFV levels in hair were measured by the UCSF Hair Analytical Laboratory using validated methods; TFV levels below the limit of quantification (BLQ) in hair, specifically <0.002 nanograms/milligrams, defined long-term non-adherence.

**Results:** The trial enrolled 49 women in the PUMA arm and 51 women in the SOC arm with counseling performed every 3 months. Retention in the study was 86% in both arms. Hair was collected and tested from 42 participants in the PUMA arm at month 12; hair was collected from 43 participants and testing completed for 41 of those samples in the SOC arm. The percent of hair samples at 12 months with TFV levels BLQ in the SOC arm was 33% versus 21% in the PUMA arm. The relative odds of long-term non-adherence in the SOC arm compared to the PUMA arm was 3.53 (95% confidence interval 1.03, 12.03, p = 0.04). Knowledge that the urine assay was being performed in the PUMA arm seemed to decrease overall non-adherence as compared to the control arm, even when the urine assay showed no TFV (Figure). One seroconversion occurred over the 12-month study in the SOC arm.

**Conclusion:** Scalable low-cost adherence interventions to increase pill-taking with oral PrEP are needed. PrEP counseling informed by a POC urine TFV test among at-risk Kenyan women on PrEP decreased overall non-adherence to PrEP.
at 12 months as determined by hair levels. The real-time urine TFV assay may have a role in improving long-term PrEP adherence.

1115 Accuracy of PrEP Adherence Measures Among Transwomen and Young MSM in Latin America: ImPrEP Study

Thiago S. Torres 1, Mayara Secco Torres da Silva 1, Carolina Coutinho 1, Pedro Leite 1, Ronald Moreira 1, Brenda Hoagland 1, Juan V. Guanira 1, Menos Benedicti 1, Hamid Vega 1, Sergio Bautista 1, Carlos Caceres 1, Peter L. Anderson 2, Beatriz Grinzigi 1, Valdilea Veloso 1, for the ImPrEP Study Group
1Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil, 2Universidade Federal do Rio de Janeiro, Brazil

Background: HIV incidence is high among young men who have sex with men (YMSM) and transgender women (TGW). PrEP is a key strategy to reduce new HIV infections, and monitoring PrEP adherence is essential to guide implementation programs. We aimed to assess the accuracy of indirect PrEP adherence measures with drug concentrations in dried blood spots (DBS) among YMSM and TGW enrolled in the ImPrEP study.

Methods: ImPrEP was an implementation project offering same-day oral PrEP for 9509 MSM/TGW in Brazil, Mexico, and Peru (Feb/2018-Jun/2021), with follow-up visits scheduled 4 weeks post-enrolment and quarterly thereafter, that included YMSM aged 18-24 years and TGW (all ages) who collected at least one DBS during follow-up. We compared two indirect adherence measures with DBS: medication possession ratio (MPR) (ratio between tablets dispensed vs. days of follow-up), and DeLong test to compare the curves. We calculated optimal cut-off points for discriminating protective drug levels based on Yuden index and their respective sensitivity, specificity, negative (NPV) and positive (PPV) predictive values.

Results: We included 4274 DBS samples from 2096 participants (week 4: 1905[44.6%], week 28: 1170[27.4%], week 52: 745[17.4%], week 76: 254[5.9%]; week 100: 135[3.1%], week 124: 65[1.5%]). Overall, 1692 (80.7%) participants were MSM and 404 (19.3%) TGW; most were aged 18-24 years (1802; 86.0%), non-white (1582; 75.5%), and had ≥ 12 years of education (1374; 65.5%). Of all DBS samples, 2871(67.2%) had protective drug levels. AUC was 0.75(95%CI:0.74-0.77) for MPR and 0.76(95%CI:0.74-0.78) for self-report adherence (Table), with no difference between adherence assessment methods’ curves (p>0.38). Calculated cut-off points for MPR and self-reported adherence were 97.0% and 93.3%, respectively.

Conclusion: Self-reported adherence and MPR adequately discriminated protective levels of PrEP among key populations in Latin America at different time points during the study follow-up. These measures are low-cost, easy to implement, and allow for immediate action to support PrEP adherence at health service level and ultimately contribute to monitoring PrEP programs.

Table: Association between indirect PrEP adherence measures and protective drug levels (TFV-DP ≥ 550 fmol/punch at week 4; ≥ 800 fmol/punch [other weeks])

<table>
<thead>
<tr>
<th>Population</th>
<th>Measures</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>MPR</td>
<td>0.77(0.74-0.79)</td>
<td>78.6</td>
<td>81.3</td>
<td>0.81</td>
<td>0.77</td>
</tr>
<tr>
<td>MPR</td>
<td>0.74(0.71-0.77)</td>
<td>80.3</td>
<td>76.8</td>
<td>0.78</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>MPR</td>
<td>0.75(0.72-0.78)</td>
<td>97.0</td>
<td>95.2</td>
<td>0.90</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>MPR</td>
<td>0.76(0.74-0.79)</td>
<td>82.1</td>
<td>85.2</td>
<td>0.88</td>
<td>0.80</td>
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</tr>
<tr>
<td>MPR</td>
<td>0.77(0.74-0.80)</td>
<td>89.4</td>
<td>87.3</td>
<td>0.92</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

1114 Point-of-Care Urine Tenofovir Test Predicts Future PrEP Discontinuation Among Young PrEP Users

Matthew A. Spinello 1, Rikki Montoya 1, Carlos Moreira 1, Karen Kuncze 1, Kevin Sassaman 1, David V. Glidden 1, K Rivet Amico 1, Emily Arnoldi 1, Susan P. Buchbinder 1, Leah Davis Ewart 1, Adam Carrió 1, Guohong Wang 1, Hideaki Okochi 1, Hyman Scott 1, Monica Gandhi 1
1University of California San Francisco, San Francisco, CA, USA, 2San Francisco AIDS Foundation, San Francisco, CA, USA, 3University of Michigan, Ann Arbor, MI, USA, 4San Francisco Department of Public Health, San Francisco, CA, USA, 5Florida International University, Miami, FL, USA, 6Abbott Labs, Abbott Park, IL, USA

Background: Young men who have sex with men (MSM) and transgender women (TGW) have both disproportionately high HIV incidence and greater challenges with PrEP persistence. POC urine tenofovir (TFV) testing permits real-time objective monitoring for non-adherence within clinical settings at a low cost. We performed urine point-of-care (POC) testing among young PrEP users (age<30) at a high-volume PrEP clinic to examine: 1) the relationship between low PrEP adherence and future PrEP discontinuation, and (2) the accuracy of POC testing vs. liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods: Participants (age<30) were recruited at the time of a daily PrEP (F/TDF or F/TAF) visit and asked to provide a urine sample and complete a survey. Adjusted logistic regression models analyzed the relationship between the primary predictor of urine POC lateral flow assay results (cut-off of 1,500 ng/mL) and the primary outcome of PrEP discontinuation, defined as no PrEP follow-up within ≥120 days of observation, given that only 90-day prescriptions were provided.

Results: Overall, the participants (n=100) had a median age of 27 (IQR 21-29) years; 12% identified as gender queer, 2% as TGW; 23% Hispanic, 20% Asian, 10% Black, 2% Native American, 2% Pacific Islander; 33% used F/TAF; 95% had at least 2 partners of unknown status. At the index PrEP visit (6/2021-5/2023), 19% had low urine TFV, and 21% discontinued PrEP without follow-up within 120 days. A low urine TFV predicted future PrEP discontinuation (AOR 6.1; 95% CI: 1.4-11; p=0.005) and was 71% sensitive and 90% specific for future discontinuation. All participants with low TFV at the index visit reported condomless anal sex with ≥2 partners of unknown status. Self-reported low adherence (<4 pills weekly) was not associated with PrEP discontinuation (p=0.18); and was only 45% sensitive and 84% specific in predicting low TFV levels. When compared to LC-MS/MS testing, POC testing was 98% sensitive/100% specific. Most (98%) wanted to be able to use the urine test on their own; 94% if it could only be administered by a clinician.

Conclusion: In a diverse sample of young MSM and transgender women using oral F/TDF or F/TAF PrEP, POC urine TFV testing predicted future PrEP discontinuation more accurately than self-report and was highly accurate when compared to LC-MS/MS. Urine POC testing can be a powerful tool for targeting PrEP adherence interventions towards those most likely to discontinue PrEP in the future.
age groups. Differences in median days on PrEP per year were assessed by gender, race/ethnicity, and age. To assess the potential impact of 2-1-1 PrEP dosing on median days of PrEP use, we compared data from 2018 (before CDC recommendation of 2-1-1 dosing) to data from 2022.

**Results:** We evaluated data from 225,180 PrEP users in 2018, and 459,984 PrEP users in 2022. In 2022, the mean number of covered days by dispensed PrEP prescriptions among PrEP users was 187 (SD: 129.8; Figure). Mean days was lower among female (median 123 days) than male (median 192 days) users (P<0.01). Among PrEP users with race/ethnicity data, mean days of use were higher for White non-Hispanic (NH) (225 days) than for Hispanic (239 days) or Black NH (231 days) users (P<0.01 for each comparison). Older users had more days covered by PrEP than younger users (<16 years: 126 days; 16-29 years: 159 days; >30 years: 201 days; p<0.01 for comparisons between <16 years and other groups). A comparison of overall data for 2018 (182 days) versus 2022 (187 days) did not suggest that the USPHS 2021 inclusion of 2-1-1 PrEP in PrEP guidelines was associated with fewer days of coverage.

**Conclusion:** PrEP programs are often evaluated by enumerating people who used PrEP at any time during a year; our data indicate that there are significant differences in PrEP coverage during a year’s time, and that an annual use indicator might mask inequities in PrEP protection, with women, Black NH and Hispanic people, and younger people having fewer dispensed days of coverage. Assessments of days of PrEP coverage should be included in assessments potential impact of PrEP and to address and monitor PrEP equity.

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**1117 Out-of-Pocket Payments for PrEP Ancillary Services Among US Commercially-Insured Persons, 2017-2021**

Ya-Lin A. Huang, Weiming Zhu, Sloane A. Bowman, Karen W. Hoover
Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** In June 2019, HIV preexposure prophylaxis (PrEP) received a grade A recommendation from the U.S. Preventive Services Task Force (USPSTF). Under the Affordable Care Act, private health plans and Medicaid expansion programs must cover the costs of all PrEP services without any patient cost-sharing starting in January 2021, including PrEP medications and ancillary services such as clinical visits and laboratory testing. The objective of this study was to monitor trends in out-of-pocket (OOP) payments for PrEP ancillary services from 2017–2021 using a large commercial claims database.

**Methods:** We analyzed data from the Merative™ MarketScan® Commercial Database that contains adjudicated medical claims. Using a validated algorithm, we identified persons aged ≥18 years prescribed PrEP from 2017–2021 and restricted the sample to those continuously enrolled in their plans for at least 6 months. We extracted all medical claims submitted for PrEP services within 1 week before each PrEP prescription using Current Procedural Terminology codes. We analyzed only fee-for-service claims and computed mean annual total and OOP (sum of copayment, deductible, and other coinsurance amounts) payments for each service. We also summed each service’s total and OOP payments for combined amounts. All payments were inflated to 2021 U.S. dollars using the medical Consumer Price Index.

**Results:** Between 2017–2021, we identified 127,055 adults prescribed PrEP. In 2021, PrEP users paid an average out-of-pocket cost of $34.60 for evaluation and management, $4.67 for preventive counseling, $3.08 for HIV testing, $2.98 for hepatitis B testing, $2.87 for hepatitis C testing, $1.30 for syphilis testing, $9.85 for gonorrhea testing, $10.42 for chlamydia testing, $1.91 for creatinine testing, and $2.84 for lipid testing. For most laboratory testing, the proportion of persons paying zero OOP payment increased over time, and more than 70% paid zero OOP payment in 2021. The mean total payment of combined PrEP ancillary services decreased from $403.97 in 2017 to $268.11 in 2021, and the mean OOP payments decreased from $89.70 in 2017 to $74.52 in 2021 (Figure).

**Conclusion:** Despite a decreasing trend in total and OOP payments for PrEP ancillary services from 2017–2021, about 30% of commercially insured PrEP users paid OOP payments after the ACA provision of no cost sharing went into effect in 2021. Efforts are needed to ensure that patient OOP payments are not required by applicable third-party payers.

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**1118 PrEP Indicators by Race/Ethnicity Among Heterosexual Women Receiving CDC-Funded HIV Testing Services**

Deeshia Patel1, Weston O. Williams2, Carolyn Wright1, Shaliondel Benton1, Merfin S. Mulatu3
1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Public Health Analytic Consulting Services, Inc., Atlanta, GA, USA

**Background:** Pre-exposure prophylaxis (PrEP) is effective at reducing the risk of HIV acquisition. However, PrEP utilization among women remains low, especially among Black/African American (hereafter referred to as Black) and Hispanic/Latina women. We examined indicators for PrEP use and PrEP-related services by race/ethnicity among heterosexual women testing negative for HIV infection via CDC-funded HIV testing.

**Methods:** We used 2019–2021 HIV testing data submitted by CDC-funded state and local health departments (n=60) and community-based organizations (n=150) to the National HIV Prevention Program Monitoring & Evaluation system. We analyzed the following indicators for heterosexual women: current PrEP use, eligibility for PrEP referral among those testing negative and not currently using PrEP, referral to a PrEP provider among those eligible, and assistance with linkage to a PrEP provider among those who received a referral. To compare each indicator by race/ethnicity, we calculated adjusted prevalence ratios (aPRs) with 95% confidence intervals (CIs) and p-values—adjusting for age, U.S. Census region, and year—with White women as the referent group.

**Results:** The prevalence of current PrEP use ranged from 0.3% to 1.1%, in adjusted models, current use was higher for multiracial (1.1%; aPR: 1.73) and Black (0.9%; aPR: 1.30) women compared to White women (0.7%; all p<0.05). Eligibility was higher for multiracial (47.2%; aPR: 1.23), Black (44.0%; aPR: 1.22), and Asian (43.3%; aPR: 1.16) women, and lower for Hispanic/Latina (32.0%; aPR: 0.90) and Native Hawaiian/Pacific Islander (33.5%; aPR: 0.89) women, versus White women (38.1%; all p<0.05). Referral was higher for American Indian/Alaska Native (50.8%; aPR: 1.59) and Black (36.4%; aPR: 1.09) women, but lower for Asian women (25.4%; aPR: 0.85), versus White women (31.7%; all p<0.05). Assistance with linkage was higher for Black women (75.8%; aPR: 1.05), but lower for Hispanic/Latina (65.0%; aPR: 0.94), Asian (60.8%; aPR: 0.88), and multiracial (63.8%; aPR: 0.93) women, versus White women (69.5%; all p<0.05).

**Conclusion:** PrEP use was low among all heterosexual women testing negative for HIV infection. PrEP-related services reached a greater proportion of Black heterosexual women; however, PrEP-related services need to reach all racial/ethnic groups, especially Hispanic/Latina women, to increase PrEP use and reduce HIV acquisition for all heterosexual women at greater risk for HIV.

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**Footnotes:**

1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Public Health Analytic Consulting Services, Inc., Atlanta, GA, USA

**Figure:** Mean annual patient out-of-pocket payments for PrEP ancillary services, 2017–2021 (2021 U.S. dollars).

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**Table:**

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>% of Women (95% CI)</th>
<th>aPR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>0.7 (0.6, 0.8)</td>
<td>1.00</td>
<td>0.50</td>
</tr>
<tr>
<td>Black</td>
<td>0.9 (0.8, 1.0)</td>
<td>1.30</td>
<td>0.01</td>
</tr>
<tr>
<td>Hispanic/Latina</td>
<td>0.7 (0.6, 0.8)</td>
<td>1.22</td>
<td>0.01</td>
</tr>
<tr>
<td>Native Hawaiian/Pacific Islander</td>
<td>0.5 (0.4, 0.6)</td>
<td>1.20</td>
<td>0.50</td>
</tr>
<tr>
<td>Asian</td>
<td>0.8 (0.7, 0.9)</td>
<td>1.16</td>
<td>0.10</td>
</tr>
<tr>
<td>Multiracial</td>
<td>1.1 (1.0, 1.2)</td>
<td>1.73</td>
<td>0.01</td>
</tr>
<tr>
<td>Alaska Native</td>
<td>0.7 (0.6, 0.8)</td>
<td>1.04</td>
<td>0.80</td>
</tr>
<tr>
<td>American Indian/Alaska Native</td>
<td>0.9 (0.8, 1.0)</td>
<td>1.59</td>
<td>0.10</td>
</tr>
</tbody>
</table>

**Note:** aPR: Adjusted prevalence ratio; CI: Confidence interval; PrEP: Pre-exposure prophylaxis; U.S.: United States.
1119 Optimizing PrEP Outcomes for MSM Who Sell Sex: The Role of Stigma, Violence, and Mental Health
Kaitlyn Atkins, John Mark Wigginton, Thomas Carpinti, Travis H. Sanchez, Stefan Baral* The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; *San Diego State University, San Diego, CA, USA

Background: Among gay men and other men who have sex with men (MSM) in the U.S., those who sell sex are disproportionately affected by HIV and report decreased uptake of pre-exposure prophylaxis (PrEP) and other HIV prevention methods. We sought to understand stigma experiences of MSM who sell sex and stigma’s role as a potential barrier to PrEP in this population.

Methods: Data were from two rounds of repeat cross-sectional online surveys of U.S. MSM (n=12,601) conducted from September 2021 through June 2023. We described stigma, violence, mental health, and PrEP experiences using chi-square tests to compare among MSM who sold sex for money, drugs, or something else in the last year to other MSM. Among MSM who sold sex who were PrEP eligible and not living with HIV, we used modified Poisson regression with robust variance to calculate prevalence ratios (PR) and 95% confidence intervals (CI) for the association between stigma and (1) maximum oral PrEP adherence (reporting 30 of 30 doses in the last month) and (2) willingness to use injectable PrEP if available. Analyses adjusted for age, race, education, and survey year.

Results: Compared to other MSM, those who sell sex reported higher levels of stigma from family and friends, general social stigma, physical violence, symptoms of post-traumatic stress disorder, and depressive symptoms (Table). Maximum daily oral PrEP adherence were lower among MSM who sell sex than other MSM (54% vs 75%, p<0.001). In adjusted analyses with PrEP-eligible MSM who sell sex, decreased PrEP adherence was associated with lifetime exposure to violence (PR 0.68, 95% CI 0.47-0.99) and social stigma (PR 0.57, 95% CI 0.33-0.96). MSM who sell sex were more willing to use injectable PrEP (57% vs 43%, p=0.001) and on-demand PrEP (75.2% vs 63.6%, p=0.004). Those willing to try injectable PrEP most preferred delivery at home (39%) or in STI clinics (20%). Increased willingness to use injectable PrEP was associated with anticipated healthcare provider stigma (PR 1.38, 95% CI 1.01-1.90).

Conclusion: Using data from over 12,000 MSM, we saw increased stigma, violence, and mental health concerns among MSM who sell sex. These issues should be concurrently addressed to optimize HIV prevention in this marginalized population. Addressing stigma and violence toward MSM who sell sex may improve adherence for those who intend to use daily. On-demand or injectable PrEP may more effectively reach MSM who sell sex if concerns about healthcare stigma are adequately addressed. The figure, table, or graphic for this abstract has been removed.

1120 Behavioral and Structural Interventions for PrEP Adherence Among Young Female Sex Workers in Kenya
Kawango Agot, Dominique M. Reed, Matthew R. Lambi, Dan Omollo, Julie Franks, Jane Moraa, Joanne E. Mantell, Allison Zerbe, Timothy Okello, Dan Omollo, Wafaa El-Sadr

ICAP at Columbia University, New York, NY, USA; New York State Psychiatric Institute, New York, NY, USA; ICAP at Columbia University, Kisumu, Kenya

Background: In Kenya, estimated HIV incidence is substantially higher among young female sex workers (YFSW) compared to similar-aged women not engaged in sex work (2.2% vs. 0.15%). Pre-exposure prophylaxis (PrEP) for HIV prevention is recommended for at-risk populations, but its effectiveness requires consistent access and adherence. We assessed the effectiveness of two behavioral and structural interventions on PrEP adherence among YFSW in Kisumu, Kenya.

Study follow-up (F/U) coincided with national restrictions on travel and gatherings due to the COVID-19 pandemic.

Methods: We conducted an unblinded, randomized-controlled trial enrolling 18-24-year-old HIV-negative YFSW with no current or recent PrEP use. Participants were provided oral PrEP and randomized to either weekly adherence support from a trained peer supporter (PS), or SMS reminders and resource transfer (RRT) for 12 months, and received PrEP with no adherence support interventions for another 12 months to assess durability of our interventions. Primary outcomes compared adherence in study arms via detectable metabolites in whole blood samples and self-report at 12, 18, and 24 months of F/U. We conducted an intention-to-treat analysis of differences in intervention effectiveness at months 12, 18 and 24. Sensitivity analyses used inverse probability weighting with stabilized weights to correct for selection bias due to lost to F/U.

Results: We screened 289 YFSW and enrolled 200 (100 per arm). Sociodemographic and sexual behavior characteristics are presented in Table 1. At 12-month F/U (n=179), 86% in PS and 93% in RRT arms reported decreases in number of weekly clients. At 12, 18, and 24 months, detectable levels of PrEP were 3%, 1%, and 0% in the PS arm compared to 9%, 9%, and 1% in the RRT (p-value = 0.4). Our sensitivity analysis found similar results. In contrast, 85%, 81% and 83% in the PS arm, and 86%, 87% and 76% in the RRT arm self-reported perfect 7-day adherence at months 12, 18 and 24, respectively. Two serocoreactions were identified; one at 12- and one at 18-months F/U.

Conclusion: In this population of YFSW, no difference in adherence by drug levels or self-report was noted across study arms. The very low levels of drug metabolites in contrast to high self-reported adherence may be due to perceived lower HIV risk resulting from decreased sex work during COVID-19 and to socially desirable responses. Findings highlight the urgent need for long-acting PrEP for this population.

Table 1: Sociodemographic and sexual behavior characteristics of study participants at baseline, by study arm

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n=200</th>
<th>PS n=100</th>
<th>RRT n=100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR)</td>
<td>22 (20-25)</td>
<td>22 (20-25)</td>
<td>22 (20-25)</td>
</tr>
<tr>
<td>Secondary education or more</td>
<td>197 (98.5%)</td>
<td>101 (100%)</td>
<td>96 (100%)</td>
</tr>
<tr>
<td>Condom use (male and female)</td>
<td>32 (16%)</td>
<td>14 (14%)</td>
<td>11 (11%)</td>
</tr>
<tr>
<td>Currently using contraceptive</td>
<td>66 (33%)</td>
<td>36 (36%)</td>
<td>30 (30%)</td>
</tr>
<tr>
<td>Number of clients in past month, Mean (SD)</td>
<td>51 (34)</td>
<td>51 (34)</td>
<td>51 (34)</td>
</tr>
<tr>
<td>Frequency of alcohol use, 1 time a week</td>
<td>31 (15%)</td>
<td>18 (18%)</td>
<td>13 (13%)</td>
</tr>
<tr>
<td>Non-alcoholic drug use, past year</td>
<td>26 (13%)</td>
<td>14 (14%)</td>
<td>12 (12%)</td>
</tr>
<tr>
<td>Depressive disorder (Axis I)</td>
<td>22 (11%)</td>
<td>12 (12%)</td>
<td>10 (10%)</td>
</tr>
</tbody>
</table>

Factors Associated With Inadequate PrEP Adherence Among TGW and Young MSM: ImPrEP Study

Institute Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil; Instituto Nacional de Infectologia Evandro Chagas (Inil, Fiocruz); Rio de Janeiro, Brazil; Ministry of Health, Brasilia, Brazil; Universidad Peruana Cayetano Heredia, Lima, Peru; Instituto Nacional de Psiquiatria Ramon de la Fuente Muñoz, Mexico City, Mexico; Instituto Nacional de Salud Publica, Mexico City, Mexico; University of Colorado, Denver, CO, USA

Background: The HIV epidemic disproportionately affects transgender women (TGW) and young men who have sex with men (YMSM) in Latin America. Monitoring adherence is key to designing tailored strategies to improve PrEP persistence, targeting these vulnerable populations. We aimed to identify factors associated with non-protective TDF-FTC levels in dried blood spots (DBS) in TGW and YMSM using PrEP.

Methods: ImPrEP was an implementation study offering same-day oral PrEP (TDF/FTC) for 9509 MSM/TGW in Brazil, Mexico, and Peru (2018-2021). Follow-up visits were scheduled 4 weeks post-enrolment and then quarterly. In this analysis, YMSM (18-24 years) and TGW (all ages) who had at least one DBS obtained during study follow-up were included. We used generalized estimating equations to identify factors associated with inadequate PrEP adherence (tenofovir diprophosphate (TFV-DP)<550 fmol/punch [week 4] or 800 fmol/punch [weeks 28-124] in DBS) among MSM/TGW (18-24 years) and TGW (all ages), separately.

Results: We analyzed 3573 DBS samples of 1,802 young participants [week 4: 1645 (46%); weeks 28-124: 1928 (54%)], and 928 DBS samples of 404 TGW [week 4: 358 (39%); weeks 28-124: 570 (61%)]. Of young participants, 94% were MSM, 14% aged 18-19 years, 75% non-white and 70% had post-secondary education. Among TGW, 27% were 18-24 years, 77% non-white and 57% had secondary education. For both populations, being from Peru and low educational level (primary or secondary education) increased the odds of inadequate PrEP adherence, while having more than 10 partners decreased the odds of inadequate adherence. Sex work and being TGW were associated with inadequate adherence among young participants, while for TGW, being 18-24 years showed the same association. Use of poppers (for young participants) and reporting condomless anal sex (for TGW) decreased the odds of inadequate adherence (Figure).

Conclusion: Sexual behavior might have influenced HIV risk perception, leading to higher PrEP adherence. Social determinants of health such as education played a major role in PrEP adherence among TGW and young MSM/TGW in Latin America. In Latin America, PrEP programs for young MSM/TGW
must implement tailored interventions to tackle stigma within health services and optimize adherence among the most vulnerable, such as those with lower education and sex workers. Long-acting PrEP can be strategical to improve PrEP adherence in these populations.

Figure: Factors associated with inadequate PrEP adherence. TFV-DP levels in DBS suggestive of <4 days of TFV-DP per week, according to characteristics of young (a) and YGW (b) participants (n=41).

1122 Alcohol Use and the Preexposure Prophylaxis Continuum of Care Among Men in Rural South Africa
Alison C. Castle1, Jacob Busang2, Jacey Dreyer1, Carina Herbst1, Nonhlanhla Okesola3, Natsayi Chimbindi2, Thembelihle Zuma2, Jana Jarolimova1, Christina Piaros1, Judith Hahn1, Sheela Shenoi1, Maryam Shahnaz1, Mark J. Siedner5
1Massachusetts General Hospital, Boston, MA, USA, 2Africa Health Research Institute, Mhlabubu, South Africa, 3University of California San Francisco, San Francisco, CA, USA, 4Yale University, New Haven, CT, USA, 5University College London, London, United Kingdom

Background: Despite freely available pre-exposure prophylaxis (PrEP), HIV incidence among young men in South Africa is high. There is conflicting evidence around the association between alcohol use behaviors and PrEP utilization. We explore the impact of hazardous alcohol use on PrEP initiation and retention among South African men.

Methods: We performed a secondary analysis of data from a trial that included men aged 16-29 randomly selected from a demographic surveillance site in KwaZulu-Natal. All participants were referred to HIV and sexual reproductive health services, where those at risk for HIV were offered oral PrEP. Alcohol consumption was assessed at monthly visits and categorized as: non-drinking (0), low/moderate risk drinking (1-5), and high/very high-risk drinking (6-12) based on AUDIT-C criteria. Primary outcomes were PrEP initiation and retention in PrEP services for >3 months. We fitted logistic regression models, adjusted for potential clinical and demographic confounders, to estimate relationships between PrEP initiation/retention and hazardous alcohol consumption.

Results: Of the 847 men referred to study clinics, 528 (62%) attended at least once. 156 were excluded due to missing data (n=107), positive HIV testing (n=20) or declined HIV testing (n=29), leaving 372 men in the analytic cohort. Average age was 22.5 years (SD 3.6) and 131 (35%) had high/very high alcohol consumption (AUDIT-C score ≥6). Men with the highest risk alcohol use also reported frequent condomless sex (89%, vs 56% in no alcohol group). We found the greatest uptake of PrEP among the high/very high-risk group (46/131, 35%), followed by the low/moderate risk group (17/63, 27%) and the no alcohol group (25/172, 17%). The high-risk group remained significantly more likely to initiate PrEP compared to the non-drinking group in multivariable models adjusted for confounders (aOR 2.02 95%CI 1.07-4.02; p-value 0.045). 30% (26/88) of men remained on PrEP at 3 months. Men with high/very high-risk drinking had similar PrEP retention at 3 months compared to men who identified as non-drinkers (aOR 1.43 95%CI 0.33-6.12; p-value 0.63).

Conclusion: High-risk alcohol use is common among men in rural South Africa and associated with increased PrEP initiation. PrEP retention was low overall, but similar across all levels of alcohol use. Hazardous alcohol use should not discourage PrEP implementation efforts to engage and retain young men.

1123 Characterizing HIV Preexposure Prophylaxis (PrEP) Discontinuation Among Men Who Have Sex With Men
Wenting Huang, Travis H. Sanchez, Marissa J. Hannah, Kelsey C. Coy, Cristian S. Acero, Aaron J. Siegler
Emory University, Atlanta, GA, USA

Background: For PrEP to have optimal impact, persons indicated for PrEP must not only initiate it but also be retained in care. However, few studies have assessed characteristics associated with PrEP discontinuation among men who have sex with men (MSM).

Methods: We conducted a descriptive analysis using data from the 2022 American Men's Internet Survey, a web-based behavioral survey of U.S. MSM. Eligible participants for this analysis were cisgender males aged ≥15 years, resided in the U.S., HIV negative, and were gay, bisexual, or had a history of sex with other men. To explore the characteristics of MSM who have discontinued PrEP, we categorized participants into three groups: persons who never used PrEP, are currently using PrEP, or have discontinued PrEP (used in the past but not currently using). We performed multivariate logistic regression, adjusting for all covariates presented.

Results: Of the 3016 participants, 2033 (67%) were PrEP naïve, 1085 (36%) were PrEP users, 50 (2%) had discontinued PrEP. PrEP discontinuation among HIV-negative persons was higher (33% vs 20%, aOR=1.81, 95%CI=1.43-2.34) and sexually transmitted infections (STIs) were more frequent (14% vs 5%, aOR=2.69 95%CI=1.86-3.90). These relationships were similar for MSM who had discontinued PrEP over a year ago and for those who discontinued PrEP within last year.

Conclusion: One in ten MSM participants in this national survey had discontinued PrEP. Risk for this group, in terms of condomless sex and STIs, was elevated relative to MSM who never initiated PrEP. Structural barriers, such as health insurance and lower educational attainment, were associated with PrEP discontinuation indicating that discontinuation may not solely be due to decreased risk. Tailored intervention is needed to support persons who have discontinued PrEP, such as health messaging, clinical discussions, and ensuring fewer barriers to care, to optimally address the sexual health needs of this group.

1124 PrEP Non-Persistence and New HIV Diagnoses: A Real-World Analysis of >120,000 People Prescribed PrEP
Li Tao, Juan Yang, J.C. Hojilla, Anand P. Chokkalingam, Christoph Carter, Moupali Das
Gilead Sciences, Inc, Foster City, CA, USA

Background: HIV pre-exposure prophylaxis (PrEP) uptake has increased, but discontinuation and inconsistent use (referred to here as non-persistence) remain common. The impact of PrEP non-persistence on new HIV infections at the population level is not well characterized. In this study, we leveraged a large real-world dataset in the United States to evaluate the impact of non-persistence on HIV diagnosis rates.

Methods: PrEP-naïve adults with at least one oral F/TDF or F/TAF for PrEP prescription dispensed between April 2021 and March 2022 were identified from the IQVIA Real-World Longitudinal Prescriptions and Diagnosis Database, a retail pharmacy claims dataset, and were followed for up to 12 months from first prescription claim submitted. Periods of PrEP non-persistence were defined as gaps in prescription claims of >30 days following the end of the calculated PrEP supply. This approach allowed for the determination of HIV diagnosis rates during periods when individuals had PrEP on-hand (on-PrEP) versus periods of off-PrEP.
Outcomes of a Community-Clinic Hybrid PrEP Trial in China During COVID Lockdowns, 2021-2023

Zhuoheng Yin1, Yifan Dai2, Chengxin Fan3, Gifty Marley4, Chunyan Li5, Songjie Wu6, Quanmin Li7, Joseph D. Tucker8, Jonathan Lio9, Hainei Huang10, Ke Liang11, Linghua Li12, Aniruddha Hazra13, Renshou Sheer14, Weiming Tang15

1Institute for Global Health and Infectious Diseases, Guangzhou, China, 2Dermatology Hospital of Southern Medical University, Guangzhou, China, 3Nanning Medical University, Nanning, China, 4University of Tokyo, Tokyo, Japan, 5Zhongnan Hospital of Wuhan University, Wuhan, China, 6Guangzhou Eight People’s Hospital, Guangzhou, China, 7London School of Hygiene and Tropical Medicine, London, United Kingdom, 8University of Chicago, Chicago, IL, USA, 9Wuhan Tongtang LGBT Center, Wuhan, China, 10University of North Carolina at Chapel Hill, Chapel Hill, NC, USA


Methods: We conducted a 12 month PrEP demonstration project in Wuhan and Guangzhou, China, using a community and clinic hybrid delivery model for recruitment, participant engagement, and PrEP delivery. Healthcare providers implemented prescribing, medical consultation, and PrEP dispensing through clinic visits or courier delivery (through community-based organizations). PrEP refill was monthly for the first quarter and trimonthly thereafter. PrEP continuation and adherence information (defined as self-reported taking more than 4 pills in 7 days for daily and over 75% strict adherence to 2+1+1 for events-driven) was surveyed quarterly. Enrollment, PrEP persistence, adherence, discontinuation, and adverse events were descriptively summarized.

Lockdowns occurred during 2021-2022 due to covid restrictions.

Results: From September 2021 to July 2023, a total of 3649 GBMSM were screened, and 1200 were enrolled. Of those, 1138 participants started oral PrEP, with a median age of 29 (IQR=5). Most participants identified as gay or bisexual (93.7%, 1066/1138), and 99.6% were cis-gender men (1134/1138).

After initiation, PrEP persistence rates at 3, 6, 9, and 12 months were 84.2% (876/1059), 75.3% (840/845), 67.1% (472/703), and 55.5% (298/537) respectively (longitudinal, 45.2% (396/878) and 54.8% (480/878) chose the daily and on-demand regimen initially. At the end, 37.2% of participants reported regimen transition (111/298) in a increasing trend (31, 41, 61, and 67 at 3, 6, 9, and 12 months). 62.0% (126/201) participants transferred from daily to on-demand regimen. The self-reported adherence rate was 75.4% (716/950), 67.8% (515/768), 58.6% (387/660), and 47.3% (237/503) at months 3, 6, 9, and 12, respectively.

239 participants (21.0%, 239/1138) discontinued PrEP use during study. Overall STIs incidence within study period is 4.9%-8%. Six participants seroconverted, resulting in an HIV incidence rate of 0.73 per 100 person-years.

Conclusion: The hybrid CBO and clinic-based model proved feasible for reaching and dispensing PrEP among Chinese at-risk populations. On-demand use and mail order drugs were popular alternatives, and one half of participants engaged in sex using alcohol and nitrates. Long-term PrEP persistence and optimal adherence continuously decreased among Chinese users during the 12-month period.
1127 PrEP Uptake and Persistence Among Incarcerated People in Zambia: Early Results From a Cohort Study

Cassidy W. Claassen1, Brianna Lindsay1, Mayuanda Siyambango1, Linah Mwango1, Caitlin Baumann1, Nasho Nyiorongo2, Gideon Daka3, Clement Moonga3, Brianna Lindsay4, Caitlin Musheke4, Michael Herce4
1University of Maryland, Baltimore, MD, USA, 2Center for Infectious Disease Research in Zambia, Lusaka, Zambia, 3University of Zambia, Lusaka, Zambia, 4Maryland Global Initiatives Corporation, Lusaka, Zambia, 5University of Maryland - College Park, College Park, MD, USA, 6University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: The time during and immediately after incarceration can be high-risk periods for HIV acquisition, particularly in sub-Saharan Africa (SSA). Incarcerated people often have very limited access to effective HIV prevention measures. In 2019, Zambia began offering HIV pre-exposure prophylaxis (PrEP) in correctional facilities. We report early results from one of the first longitudinal studies of PrEP uptake and persistence in a correctional setting in SSA.

Methods: In August 2023, we launched a cohort study of PrEP users and non-users in three correctional facilities in Lusaka, Zambia. Following HIV testing, HIV-negative incarcerated persons were offered PrEP by the corrections health system, and then were approached by study staff for participation. Consenting participants were followed from incarceration through release into the community for PrEP outcomes, including uptake, persistence, and adherence. A subset of participants on PrEP underwent urine tenofovir (TFV) screening for adherence, and another subset of both PrEP users and non-users will be consented to participate in qualitative interviews in October 2023.

Results: From 8 August to 21 September 2023, we screened 124 incarcerated persons; 120 (97%) were eligible and consented. Participants were 18–52 years old, majority (84%) male, less than half (36%) completed secondary education, and three (3%) indicated previous incarceration. Of the 120 enrolled, 53 (78%) initiated PrEP while 27 (22%) declined. As of 21 September, 39 were eligible for one-month study follow-up and 74 (69%) had completed a visit, all in a correctional facility. Of these, 22 (29%) had initiated PrEP at enrollment and all were HIV-negative at follow-up. Two (9%) who had initiated PrEP at enrollment chose to discontinue PrEP at follow-up. Two of 7 (29%) who had not initiated PrEP at enrollment chose to initiate at follow-up. Of the 22 who initiated PrEP at enrollment and had ≥1 follow-up visit reported, 13 (59%) were randomized to the TFV screening sub-cohort and tested. Of these, 62% (8/13) demonstrated TFV metabolites consistent with adherence.

Conclusion: Early results suggest high demand for PrEP among incarcerated persons in Zambia. This is one of the first observational studies of PrEP uptake, persistence, and adherence among incarcerated persons, and may have future implications for HIV prevention efforts in this population.

1128 Social Determinants of Health and PrEP Uptake in the US: Repeated Measures Correlational Study

Hollie David1, Alan Wells, Susan J. Little, Sanjay R. Mehta, Thomas Martin
1University of California San Diego, La Jolla, CA, USA

Background: Pre-exposure prophylaxis (PrEP) is a cornerstone of the United States (US) Ending the HIV Epidemic plan. PrEP use was evaluated in jurisdictions with highest HIV diagnosis rates to address disparities at state and county levels on national scale.

Methods: PrEP data from 2012-2019 was analyzed using linear mixed methods modeling. Within the model, dependent variables were county and state PrEP rates. Fixed effects were year, new HIV diagnoses rates, mean income, population insurance coverage, proportion of democratic presidential candidate votes in 2020 and race, while random effects were states and counties. Public health data was from AIDSvu; demographic data from US Census Bureau ACS; 2020 presidential election results from MIT.

Results: County Level: Beginning in 2012, each sequential year was associated with increased PrEP use by 7.2/100,000, 95%CI [2.8, 11.8]. For each $1,000 increase in mean income, there was increase in PrEP use by 65.7/100,000 persons, 95%CI [47.7, 83.3]. Increased presidential election votes for the democratic candidate were associated with increased PrEP by 633/100,000 for each percentile point increase in proportion of votes, 95%CI [314, 999]. Higher HIV diagnosis rates were associated with decreased PrEP use by -1.9/100,000 for each new diagnosis per 100,000 in the population, 95%CI [-2.8, -0.9]. Jurisdictions with higher proportion of Asians were associated with decrease in PrEP by -1991/100,000 for each percentile point increase in population proportion, 95%CI [-3357, -632]. State Level: For each $1,000 increase in income, there was increased PrEP use by 45.9/100,000 persons, 95%CI [24.1, 67.6]. Increased proportion of presidential election votes for the democratic candidate were associated with decrease in PrEP by -126/100,000 for each percentile point increase in proportion of votes, 95%CI [-218, -34]. Higher insurance coverage was associated with decreased PrEP by -0.54/100,000 for each new insured person per 100,000, 95%CI [-1.0, -0.08]. States with higher proportion of Asian persons had decreased PrEP use -307/100,000 for each percentile point increase in the population, 95%CI [-4520, -1637]. States in the Midwest were had higher PrEP uptake by 18.4/100,000 95%CI [8.63, 28.3] when compared to the South as reference.

Conclusion: Region and demographic data display associations of regional characteristics with inequitable PrEP rates. Displayed variances between state and county levels may be accounted for by differences in HIV preventative medicine policy.

1129 Association of US Medicaid Expansion and Number of Persons Prescribed PrEP, 2017-2021

Karen W. Hoover, Weiming Zhu, Sheila Salvant Valentine, Ya-Lin A. Huang

Background: To accomplish goals of the Ending the HIV Epidemic in the U.S. initiative, increased PrEP uptake is needed especially in jurisdictions with higher HIV incidence. Expanded Medicaid eligibility and enrollment can provide PrEP financial access for persons who otherwise might not be able to afford its cost. The Affordable Care Act of 2010 included a provision for states to expand Medicaid coverage starting in 2014. Since then, 41 states and the District of Columbia have expanded Medicaid access. Our objective was to estimate the association between state Medicaid expansion and the use of PrEP.

Methods: We analyzed national Medicaid data from the Centers for Medicare and Medicaid Services to estimate the PrEP-to-Diagnosis Ratio (PDR) among persons aged 16 years and older from 2017–2022. The PDR is a measure of PrEP coverage that estimates the need for increased PrEP implementation. It was calculated as the annual number of persons prescribed PrEP divided by the annual number of new HIV diagnoses. We identified persons prescribed PrEP using a validated algorithm with diagnostic and drug codes. We identified persons with newly diagnosed HIV using ICD diagnosis codes for HIV. We categorized states and the District of Columbia in three cohort groups based on the year they expanded Medicaid: before 2017, 2017–2021, and never. We calculated the estimated annual percentage change (EAPC) and 95% confidence intervals (CIs) for PDR trends by state Medicaid expansion cohort.

Results: Among persons with Medicaid, the overall number of persons prescribed PrEP increased from 24,279 in 2017 to 53,434 in 2021. Among 32 states that expanded Medicaid before 2017, the PDR was 1.6 in 2017 and increased to 5.3 by 2021 with an EAPC of 35.3 (95% CI 35.3, 35.3) (Figure). Among 7 states that expanded Medicaid in 2017 through 2021, the PDR was 0.2 in 2017 and 2.8 by 2021 with an EAPC of 83.8 (83.3, 84.2). Among 12 states that did not Medicaid before 2022, the PDR was 0.2 in 2017 and 2.1 by 2021 with an EAPC of 91.0 (90.9, 91.3).

Conclusion: Our study found that expansion of Medicaid was associated with increased PrEP coverage among persons with Medicaid insurance, suggesting that Medicaid expansion is an effective policy to increase access to HIV prevention services. Medicaid expansion can provide access to many services, including PrEP, that protect the health and wellbeing of the U.S. population.
1130 A Matter of Time: Factors Associated With Delayed nPEP Initiation
Nicholas Brian Bana, Massimo Puoti, Chiara Baiguer, Alessandro Raimondi, Leonardo Rezzonico, Francesco Paracchini, Cristina Moioli, Leonardo Chianura, Giovanna Travi, Carolaardrea Orcese, Fulvio Crippa, Carlotta Rogati, Marta Vecchi, Marco Merli, Roberto Rossotti
ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

Background: Nonprofessional Post-Exposure Prophylaxis (nPEP) protects against HIV infection after risk exposure, but a prompt start is essential. According to the Italian guidelines, nPEP should be started within 24 hours after the exposure (preferably in the first 4 hours), but can be prescribed up to 72 hours. Aim of this study is to describe factors associated to users’ presentation time to our Emergency Department (ED) asking for nPEP.

Methods: Retrospective Monocentric Observational study including all individuals who consecutively accessed our ED asking for nPEP between January 2011 and July 2023. We collected demographic data, information about type of exposure, sexual orientation and additional risky behaviours, eventual previous nPEP courses or HIV testing, and presentation time to ED. Descriptive statistics and nonparametric tests were used to describe study population. Unadjusted and adjusted binary regression analyses were performed to test factors associated to an early (within 24 hours) presentation.

Results: The analysis included 522 persons who accessed ED asking for nPEP. 486 (93.1%) were males, 354 (67.6%) MSM, 97.2% of them declared sexual intercourse as risk exposure. Median presentation time overall between biological exposure and ED presentation was 14.7 hours: 519 (99.4%) individuals accessed to our ED within 72 hours, 391 (74.9%) in the first 24 hours but only 111 (21.3%) within 4 hours (Figure 1). Median waiting time in ED before nPEP start was 1.4 hours. Multivariate binary regression analysis found that Italian nationality (OR 2.04, 95% CI 1.06-3.91, p=0.032), semen/ano-genital direct contact (OR 2.09, 95% CI 1.21-3.59, p=0.008) and previous HIV testing (OR 3.00, 95% CI 1.48-6.07, p=0.002) were significantly associated with presentation within 24 hours after exposure, while sexual intercourse under the effect of alcohol or recreational drugs was associated with late presentation after 24 hours (OR 0.33, 95% CI 0.15-0.73, p=0.006). No significant effect was detected for type of sexual intercourse, sexual orientation, HIV status of source individual, and previous nPEP courses.

Conclusion: The majority of nPEP users accessed the ED within 24 hours after risk contact, even if they had to wait often more than 1 hour for nPEP prescription. Use of alcohol and recreational drugs (including Chemsex practices) during sexual intercourse represents an important risk factor for HIV acquisition not only in terms of dangerous exposure, but also for delayed nPEP start.

1132 Efficacy of Long-Acting Cabotegravir and Rilpivirine for PEP in a Macaque Model of RT SHIV infection
Priya Srinivasan, Jinping Zhang, Tiancheng Edwards, Chuong Dinh, Ayanna Green, Dawn Little, Maria Mendoza, Yi Pan, Frank Deyounks, Ryan Johnson, Athena Kouritis, Walid Heneine, Gerardo Garcia-Lerma, James Smith
Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Current recommendations for post-exposure prophylaxis (PEP) for non-occupational HIV exposures include 28 days of daily oral antiretroviral drugs. However, low adherence, and inadequate regimen completion represents an important challenge. We examined whether a single injection of the combination of long-acting cabotegravir and rilpivirine (CAB LA and RPV LA) could be an effective PEP regimen in macaques.

Methods: Human equivalent doses of CAB LA (50 mg/Kg) and RPV-LA (100 mg/Kg) were administered intramuscularly to 6 rhesus macaques 24 hours post rectal exposure to a single high dose of RT SHIV (10^5.3 TCID50). Infection outcome was compared to 7 untreated controls (3 real-time, 4 historical). Blood was collected through 43 weeks post challenge to monitor for plasma CAB and RPV levels and SHIV infection. Plasma CAB and RPV levels were monitored by LC-MS/MS. Poisson regression with robust error variance was applied to estimate PEP efficacy and confidence intervals.

Results: Median concentrations of CAB LA (50 mg/Kg) and RPV-LA (100 mg/Kg) were administered intramuscularly to 6 rhesus macaques 24 hours post rectal exposure to a single high dose of RT SHIV (10^5.3 TCID50). Infection outcome was compared to 7 untreated controls (3 real-time, 4 historical). Blood was collected through 43 weeks post challenge to monitor for plasma CAB and RPV levels and SHIV infection. Plasma CAB and RPV levels were monitored by LC-MS/MS. Poisson regression with robust error variance was applied to estimate PEP efficacy and confidence intervals.

Results: Median concentrations of CAB LA (50 mg/Kg) and RPV-LA (100 mg/Kg) were administered intramuscularly to 6 rhesus macaques 24 hours post rectal exposure to a single high dose of RT SHIV (10^5.3 TCID50). Infection outcome was compared to 7 untreated controls (3 real-time, 4 historical). Blood was collected through 43 weeks post challenge to monitor for plasma CAB and RPV levels and SHIV infection. Plasma CAB and RPV levels were monitored by LC-MS/MS. Poisson regression with robust error variance was applied to estimate PEP efficacy and confidence intervals.
309-1033 SHIV RNA copies/ml) when CAB and RPV were low or undetectable in plasma and seroconverted at week 43 (Figure 1). 

**Conclusion:** We observed in our high dose rectal SHIV challenge model that a single dose of CAB LA and RPV LA given 24 hours after virus exposure provided clinical drug exposures for 4 weeks but was partially effective. Late and transient detection of SHIV RNA in breakthrough infections with or without seroconversion is similar to findings in persons failing prophylaxis with CAB LA and highlights diagnostic challenges of this PEP modality. Our results underscore the limitations of single-dose CAB LA and RPV LA use for PEP in humans.

1133 **PrEP Following PEP: An Effective HIV Risk-Reduction Strategy**

**Gary Whitlock, Courtney Taylor, Lucy Turner, Holly Thompson**

**Chelsea and Westminster NHS Foundation Trust, London, United Kingdom**

**Background:** From January 2021, individuals attending a sexual health service in London, UK who receive HIV post-exposure prophylaxis following sexual exposure (PEPSE) are offered quick-start opt-out PrEP with a 1-month supply to take immediately following completion of PEP. PEP2PrEP, a risk reduction strategy for individuals with ongoing risk of HIV acquisition. We present the uptake of PrEP in GBMSM and transwomen attending our service for PEP and their subsequent PEP follow-up.

**Methods:** We performed a case note review of PEPSE recipients at our service from 1st March to 30th April 2022, collecting demographics, characteristics of the PEPSE risk, previous PEP use and follow-up consultations up to 31st August 2023. Statistical analysis was done using chi-square and Mann-Whitney U tests.

**Results:** 282 GBMSM and 6 transwomen received PEPSE during March-April 2022. Median age was 29 y (IQR: 25-37 y). Primary PEPSE indication was condomless anal intercourse: receptive (244, 84.7%) and insertive (43: 14.9%) and receptive vaginal intercourse (1: 0.3%). During the encounter, 31 (10.8%) used chems, 63 (21.9%) had sex with more than one individual. 126 (43.8%) PEPSE recipients stated previous PrEP use. Common reasons for not using PrEP were: having no supply (38, 30.2%), on break (30, 23.8%), spontaneous sex (19, 15.1%), incorrect PEP dosing requiring PEPSE (16, 12.7%), reason not given (23, 18.3%). 212 (73.6%) subsequently started PrEP. Of these, 114 (54.2%) reattended for a subsequent PEP consultation in the follow-up period. PEPSE users who subsequently started PrEP compared with those who did not were more likely to have used PEP previously (50.0% vs. 26.3%, p=0.00036) and to have had sex with multiple individuals during their PEPSE exposure (25.0% vs. 13.2%, p=0.036).

**Conclusion:** Almost half of PEPSE recipients have previously used PrEP. The most common reason for not using PrEP was having no supply. In PEPSE recipients, the subsequent uptake of PrEP is high with a majority reattending for PrEP in the subsequent year. Efforts to increase retention in PrEP care are required for those with ongoing risk of HIV acquisition.

1134 **BIC/FTC/TAF as HIV PEP Was Well-Tolerated With High Adherence and No Seroconversions**

**Darrell H. Tan**, Reva Persaud, Atia Qamar, Isaac I. Bogoch, Arlene Chan, Allison Chris, Karla Fisher, Richard T. Lester, John Maxwell, James Murray, Hong Qian, Hubert Wong

1StMichael’s Hospital, Toronto, Canada; 2Scarborough Health Network, Toronto, Canada; 3University Health Network, Toronto, Canada; 4Women’s College Hospital, Toronto, Canada; 5Toronto Public Health, Toronto, Canada; 6University of British Columbia, Vancouver, Canada; 7AIDS Committee of Toronto, Toronto, Canada; 8Ontario Ministry of Health and Long-Term Care, Ontario, Canada; 9Cambridge HIV Trials Network, Vancouver, Canada

**Background:** Integrase inhibitor-based regimens are the standard of care for HIV post-exposure prophylaxis (PEP), but no such single tablet regimens are recommended in current guidelines. We analyzed tolerability, adherence and HIV seroconversions with bictegravir, emtricitabine and tenofovir alafenamide (BIC/FTC/TAF) as HIV PEP in an ongoing clinical trial of text-messaging support versus standard of care for PEP users.

**Methods:** Adults initiating a standard PEP regimen within the preceding five days for a confirmed or potential sexual exposure to HIV were randomized to either receive short message service (SMS) check-ins using the WeTel platform, or standard care. All participants underwent baseline HIV testing and were switched from their original PEP regimen (if applicable) to B/F/TAF to complete 28 days. CBC, ALT and creatinine were assessed at week 2; medication adherence at week 4; HIV serology at weeks 6 and 12; and adverse events at all visits.

**Results:** Of 120 individuals screened for participation in the trial, 119 participants were enrolled and are included in this analysis; all were HIV-negative at baseline. Median (interquartile range) age was 29 (25, 34) years and 22% had previously used PEP. Most (86%) were men who have sex with men. Medication adherence was high; among 101 participants with available data, all took all 28 days of PEP except for two who stopped prematurely after 7 and 8 days respectively. B/F/TAF was well-tolerated, with only 11% experiencing adverse events of grade ≥2 severity; 38% experiencing AEs at least possibly related to study drug (Table), most often gastrointestinal. No HIV seroconversions were observed.

**Conclusion:** B/F/TAF PEP was associated with high tolerability, high adherence and no HIV seroconversion in this cohort. These data support the use of this single tablet regimen as HIV PEP after sexual exposures.

**Table: Adverse events occurring in ≥5% of participants receiving B/F/TAF PEP**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>h (% of participants)</th>
<th>% of participants</th>
<th>% of participants</th>
<th>% of participants</th>
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</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>12 (11.2%)</td>
<td>14 (12.7%)</td>
<td>4 (3.6%)</td>
<td>4 (3.6%)</td>
</tr>
<tr>
<td>Flatulence</td>
<td>10 (9.3%)</td>
<td>11 (9.8%)</td>
<td>5 (4.4%)</td>
<td>6 (5.4%)</td>
</tr>
<tr>
<td>Headache</td>
<td>5 (4.7%)</td>
<td>6 (5.4%)</td>
<td>5 (4.4%)</td>
<td>5 (4.4%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>4 (3.8%)</td>
<td>5 (4.4%)</td>
<td>3 (2.7%)</td>
<td>3 (2.7%)</td>
</tr>
<tr>
<td>Rash</td>
<td>2 (1.9%)</td>
<td>2 (1.8%)</td>
<td>1 (0.9%)</td>
<td>1 (0.9%)</td>
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<tr>
<td>Urticaria</td>
<td>2 (1.9%)</td>
<td>2 (1.8%)</td>
<td>1 (0.9%)</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (0.9%)</td>
<td>1 (0.9%)</td>
<td>1 (0.9%)</td>
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</table>

1135 **Predictors of PEP Uptake in the SEARCH Dynamic Choice HIV Prevention Trials**

**James Ayieko**, Laura B. Balzer, Elijah Kakande, Jane Kabambe, Collette Ancos, Gabriel Chanie, Catherine A. Koss, Elizabeth Bukusi, Moses R. Kampa, Maya L. Petersen, Diane Havlir

1Kenya Medical Research Institute, Nairobi, Kenya; 2University of California Berkeley, Berkeley, CA, USA; 3Infectious Diseases Research Collaboration, Kampala, Uganda; 4Kenya Medical Research Institute, Kilifi, Kenya; 5University of California San Francisco, San Francisco, CA, USA; 6Makerere University College of Health Sciences, Kampala, Uganda

**Background:** Post-exposure prophylaxis (PEP) remains underutilized in sub-Saharan Africa. PEP could respond to user preferences for on-demand biomedical prevention, including for vaginal exposures, and could serve as a gateway to PrEP; data are limited on this strategy.

**Methods:** We assessed PEP uptake in 3 randomized trials of Dynamic Choice HIV Prevention (DCP) among persons >15 years with current or anticipated HIV risk recruited from Outpatient Departments (OPD), Antenatal Clinics (ANC), and community settings in rural western Kenya and Uganda (SEARCH; NCT04810650). The DCP intervention included structured participant choice of: 1) PEP or PrEP, with option to change over time; 2) service location; 3) HIV self-testing (HVST) option, together with 24/7 phone access to clinician. Providers were trained on client-centered care, emphasizing support for flexible product choice (PrEP or PEP). In the DCP arm, PEP pill-in-pocket was offered (5 pills with HVST) for rapid PEP start; participants notified the provider for HIV testing and refill of the remaining PEP supply. In this pre-specified secondary analysis, we conducted a by-arm comparison of self-reported PEP use and describe use patterns across these settings.

**Results:** From April-July 2021, 1,233 participants (612 DCP, 621 standard of care (SoC)) enrolled (ANC 400, OPD 403, Community 430): 72% were women, 41% aged 15-24 years. Over 18 months of follow-up, 129 courses of PEP were dispensed. In DCP arm: 101 PEP courses among 50 participants; 28 participants used multiple PEP courses; 10 transitioned to PrEP the month after PEP start. In SoC: 28 PEP courses among 20 participants; 8 participants used multiple PEP courses; 2 participants transitioned to PrEP the month after PEP start. Biomedical covered time (% of follow-up time when using PEP): 1.1% (DCP) vs. 51% (SoC); difference of 0.8% (95% CI: 0.1-1.5%) p=0.03. Among DCP participants, PEP covered time was highest in the community (2.2%), men (1.8%) and youth (1.1%). There were no discontinuations for drug toxicity or seroconversions among those who used PEP.
Conclusion: Across 3 settings in rural Africa, PEP was feasible to deliver, a desired choice for some individuals, and a gateway to PrEP. Event-driven interventions such as PEP should be included in prevention choice approaches.

**1136 CAPRISA 018 Trial: Acceptability of Tenofovir Alafenamide Implants for PrEP in African Women**

Tanuja N. Gengia, Craig J. Heckt, Lara Lewis, Leila E. Mansoor, Ishana Harkoo, Diana Chetty, Nafibole Myeni, Marc M. Baumann, John A. Moss, James F. Rooney, Catherine Hankins, Bruno Pozzetto, Quarraisha Abdool Karim, Salim S. Abdool Karim

*Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, Columbia University Medical Center, New York, NY, USA, Oak Crest Institute of Science, Monrovia, CA, USA, Gilead Sciences, Inc, Foster City, CA, USA, Amsterdam Institute for Global Health and Development, Amsterdam, Netherlands, Centre International de Recherche en Infectiologie, Lyon, France*

**Background:** In South Africa, young women carry a disproportionately higher HIV risk. Long-acting HIV pre-exposure prophylaxis (PrEP) methods may better align with women’s preferences, behaviors, and lifestyles—potentially improving PrEP uptake, adherence, and persistence. We studied the acceptance of implants for PrEP in women participating in a tenofovir alafenamide (TAF) implant trial.

**Methods:** CAPRISA 018, a Phase I trial, assessed the safety and efficacy of sub-dermal TAF implants for PrEP. After receiving 1 or 2 TAF (110 mg) or placebo implants (4:1 randomization), participants (N=30) completed longitudinal (0-52 weeks) acceptability assessments on product attributes (implant size, quantity, insertion site, palpability, visibility), physical experiences (insertion procedures, pain, reactions/side effects, scarring), removal procedures, and method likes and dislikes. We averaged scores (range: 1-6, increasing with acceptability), reported minimum temporal means (min–max values) to assess acceptability (scores >4), and performed sub-group analyses by removal timing (scheduled [Week 48] vs. early). For early removals, acceptability was assessed 4 weeks post removal.

**Results:** Participants were young (median age: 26 years) Black African women. Overall pre-removal product attributes (5.4 [3.6-6.0]) and physical experiences (4.9 [1.7-6.0]) remained acceptable over time (Figure 1). On average, women with scheduled removals had high levels of acceptability for product attributes and physical experiences during the study and 4 weeks after implant removal. Early removals occurred on average 4 months (0-8 months) after implant insertion among 11 (37%) of women. Women with early removals, on average, reported acceptable pre-removal and post-removal product attributes and physical experiences; however, all reported unacceptable levels of side effects at least once during the study (100% in early removals versus 47% in scheduled removals, p=0.004). Both groups reported that implants were acceptable as a potential long-term PrEP method (5.3 [3.6-6.0]), and the most-cited reason for liking the implants was possibility of long-term HIV protection.

**Conclusion:** Although women in the trial reported high levels of acceptability for implant attributes, physical experiences, and insertion and removal procedures overall, higher than expected early discontinuation rates due to side effects were observed. The implants potential for long-term HIV protection was at least once during the study (100% in early removals versus 47% in scheduled removals, p=0.004). Both groups reported that implants were acceptable as a potential long-term PrEP method (5.3 [3.6-6.0]), and the most-cited reason for liking the implants was possibility of long-term HIV protection.

**1137 Safety and Pharmacokinetics of Ultra-Long-Acting Dolutegravir In-Situ Forming Implant**

Thy Le, Isabella C. Young, Craig Sykes, Amanda P. Schauer, Mackenzie Cottrell, Angela D. Kashuba, Sahima Benhabbour

University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

**Background:** Long-acting injectables (LAIs) for HIV PrEP can increase user acceptability, adherence, and reduce stigma. Current LAIs are not removable and elicit a long pharmacokinetic (PK) tail. In previous studies, investigational LAL Dolutegravir (DTG) formulations failed to sustain plasma DTG concentrations above the 4× PA-IC50 beyond two months. To overcome these limitations, we propose to develop an ultra-long-acting (ULA) biodegradable and removable in-situ forming implant (ISFI) with DTG. Here, we performed long-term safety and PK studies evaluating time-to-completion (ongoing), depot removal at various intervals, PK tail, and drug biodistribution in plasma, tissues, and organs post implant removal.

**Methods:** ISFIs were comprised of high concentration DTG (350 mg/mL) in a stable suspension. Female BALB/c mice (n=6) were injected subcutaneously (SC) with 30 µL of DTG ISFI formulation. Plasma samples were collected longitudinally to quantify drug concentration and TNF-α and IL-6 levels over ≥180 days. At day 30, 90, and 180 post administration, implants were removed via a small skin incision to quantify residual DTG and determine implant degradation over time. Plasma samples were collected longitudinally post-implant removal to assess the PK tail. Biodistribution of DTG in plasma, organs, and SC tissue was assessed post implant removal.

**Results:** ULA DTG ISFIs sustained plasma DTG concentrations well above the 4× PA-IC50 for ≥180 days (Fig A) and with low plasma TNF-α and IL-6 concentrations. Significant decrease in plasma DTG was achieved within 1 week post implant removal with 17-, 22-, and 24-fold decrease for implants removed at 30, 90, and 180 days respectively; however, complete elimination of DTG was not yet achieved post depot removal (Fig B). Tissue DTG concentrations revealed high DTG accumulation in the proximal SC tissue as a likely explanation of the long PK tail. ISFIs were easily retrievable at 30, 90, and 180 days post-injection with 69%, 45%, and 41% DTG remaining and 13%, 49%, and 58% decrease in implant mass at 30, 90, and 180 removal respectively.

**Conclusion:** Here we report a biodegradable, removable, and ULA DTG ISFI and demonstrated safety and plasma PK for ≥180 days and assessed the PK tail. We demonstrated the first ULA injectable of DTG for HIV PrEP that can be removed if needed. This comprehensive study further characterized the PK tail via a full biodistribution, and future directions include safety, PK and efficacy studies in non-human primates.

**1138 RCT of Lactobacillus crispatus CTV-05 to Lower HIV Risk in Young South African Women**


*University of California San Francisco, San Francisco, CA, USA, Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, University of Kwazulu-Natal, Durban, South Africa, The Aurum Institute, Johannesburg, South Africa, Ovit, Inc, Mountain View, CA, USA*

**Background:** Absence of vaginal lactobacilli and corresponding genital inflammation is associated with adverse reproductive health outcomes, including female HIV acquisition. In women in sub-Saharan Africa, a high prevalence of vaginal dysbiosis may offer an opportunity to develop Lactobacillus crispatus biotherapeutics for HIV prevention.

**Methods:** We conducted a randomized, placebo-controlled, trial of cis-women age 18-23 with Nugent score 4-10 on vaginal Gram stain who completed a course of oral metronidazole (MTZ). Women were randomized (2:1) to use vaginal applicators containing 2X10^8 CFU of L. crispatus CTV-05 (LACTIN-V) or placebo for 4 weeks. Vaginal samples were collected prior to MTZ, at enrollment...
(0-wks), immediately after study product (4-wks), and 4 weeks later (8-wks). The vaginal microbiota was assessed by 16S rRNA gene sequencing, endocervical immune cells by flow cytometry and vaginal fluid inflammatory markers by Luminex.

**Results:** 45 black South African women were randomized to LACTIN-V (N=32) and placebo (N=13). At enrollment, immediately after MTZ, 54.8% (LACTIN-V) vs. 66.7% (placebo) presented with Lactobacillus-dominant microbiota other than L. crispatus, most often L. iners (Figure). A L. crispatus-dominant microbiome was identified in 40.6% (4-wks) and 25.8% (8-wks) of participants in the LACTIN-V arm compared to 0% and 9%, respectively in the placebo arm (4-wks: p = 0.047; 8-wks: p = 0.009). The proportion of activated HIV target cells (CD8+ /CD4+ /CD38+ /HLA-DR+ /CCR5+ + T cells) within the total T cell population (CD3+) increased from post-MTZ (0-wks) to 4-wks in the placebo arm (0.38 log10 fold change) which was not observed in the LACTIN-V arm (0.03 median log10 fold change; p = 0.009). The change in the cytometry markers between pre-MTZ and 4-wks was not statistically different by arm. Three quarters of participants in both arms “strongly agreed” or “agreed” they would use the product again. Adverse events (AEs) occurred in 77.8% of all participants. All local solicited AEs, most commonly vaginal discharge, were grade 1 with no significant difference by arm.

**Conclusion:** LACTIN-V after MTZ increased L. crispatus colonization. Women treated with LACTIN-V had fewer activated cervical CD4+ HIV target cells than those in the placebo arm. We did not observe statistically significant differences in vaginal cytokines between arms. The product was safe and highly acceptable (clinicaltrials.gov:NCT05022221).

### A Dual Prevention Pill for HIV and Pregnancy Prevention: A Pilot Study Among Young Women in Zimbabwe

**Barbara A. Friedland**, Brady Teinan, Sanyuka Mathur, Irene V. Bruce, Adlight Mandadi, Peina Musarach, Coraline Moonembedi, Lisa B. Haddad, Nyaradzo Mgodi, Eva Matiko

**Background:** Effective use of oral pre-exposure prophylaxis (PrEP) has been sub-optimal among young women in sub-Saharan Africa at greatest risk of HIV. We hypothesized that a single dual prevention pill (DPP) combining PrEP and an oral contraceptive (OC) would be preferred, acceptable and increase PrEP adherence compared to PrEP alone.

**Methods:** We enrolled 30 HIV-uninfected, non-pregnant, 16-24-year-old cisgender females currently using OCs in a crossover study in Harare, Zimbabwe, who were randomized (1:1) to the order of using an over-encapsulated DPP and 2 separate pills (PrEP, OC) for 3-28-day cycles each. We assessed if the proportion preferring the DPP vs 2 separate pills was >0.5 (exact binomial test) and the effect of regimen on 4 acceptability domains: use attributes, product attributes, side-effects, impact on sex (Willcoso signed-rank tests). We compared adherence by regimen and sequence (generalized estimating equations) via self-report and via tenofovir diphosphate (TFV-DP) levels in dried blood spots indicative of ≥4 doses per week (≥500 fmol/punch at Month 1, 700 fmol/punch at Months 2-6). Population Council Institutional Review Board and 6 ethics committees/regulatory bodies in Zimbabwe approved the protocol.

**Results:** 26/30 women (mean age, 19.4 years) completed the study (Nov 2022-Sep 2023); 4 terminated early. Most were married (47%)/divorced (27%), 97% had completed secondary school, 93% had ≥ 1 child, 84% were somewhat/very worried about getting HIV in the next 3 months, and 93% thought it was somewhat/very important to avoid pregnancy. At study exit, preference for the DPP was 64% vs 36% for 2 separate pills (p = 0.16). Most rated both regimens as acceptable, with no differences between regimens in any domain (all p > 0.05).

**Self-reported adherence was high (≥96%), yet 17-18% were adherent to the 2 pills and DPP, respectively, based on TFV-DP levels, with mean TFV-DP levels only 384 fmol/punch (2 pills) and 392 fmol/punch (DPP). There was no difference in adherence by regimen or treatment period (all p > 0.05). Adherence was higher overall among participants starting with DPP vs those starting with 2 pills (not significant, Fig 1).

**Conclusion:** While we did not find differences between the DPP and 2 separate pills for any outcome, this small study used an over-encapsulated pill as a proxy for the co-formulated DPP. A larger study with the actual DPP — a smaller pill — will be a better indicator of the potential impact of the DPP on HIV and pregnancy prevention in this population.
services. THPS will continue to work with R/CHMT to strengthen and expand implementation of motorcycle enabled health workers

1141 Enhancing HIV PrEP Coverage Through Primary Care Initiation: A French Nationwide Study
Sophie Bamouni1, Sophie Billioti De Gage1, David Desplas2, Julie Valbouquet1, Julie Lamant3, Jean-Philippe Joseph1, François Dabis1, Agnès Viot1, Salim Fakri4, Rosemary Dray-Spira5, Michel Carles1
1Nice University Hospital, Nice, France, 2EHP-PHASPE, Saint-Denis, France, 3Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France, 4University of Bordeaux, Bordeaux, France

Background: In France, until mid-2021, as HIV pre-exposure prophylaxis (PrEP) initiation (PrEPi) was limited to hospitals and sexual health centers, access to PrEPi remained mainly limited to socio-economically advantaged MSM living in large urban areas. To improve access to PrEP, PrEPi has then been extended to any practitioner, including primary care (PrC) practitioners. The aim of this study was to describe the deployment and characteristics of the PrC PrEPi in the French health system.

Methods: Using the National Health Data System (SNDS) covering healthcare reimbursements of 99% of French residents, we included all people >15 years-old, receiving a PrEPi in a PrC setting from 06/01/21 to 12/31/21. Monthly numbers of PrEPi over the period, characteristics of PrEP initiators and PrEPi modalities (including prescribers profile and assessment of biological tests) and renewals were reported.

Results: Overall 13,500 PrEPi were done in PrC during the study period. The mean number of PrEPi increased from 654/month (2nd semester of 2021) to 783/month (2nd semester of 2022). PrEPi initiators were predominantly men (96.3%), mean age 36 years, mostly living in large urban areas (72.0%). A minority (7.5%) were socio-economically disadvantaged. Among PrEPi prescribers, 88.0% were general practitioners, 77.0% had a fully private practice and 44.6% were the patient’s family practitioner. Reimbursements for HIV, renal and liver function tests, 60 days before to 30 days after the first PrEP dispensation, were available for 72.5%, 66.8% and 54.7% of PrEPi, respectively. Syphilis, chlamydia or gonorrhea screenings were reimbursed for 64.7% and 59.7% of PrEPi, respectively. In the six months post PrEPi, 70.8% of PrEP initiators had at least one renewal (2.3 renewals on average). Most of them (80.0%) had the PrEPi renewal by the PrEPi prescriber, especially when the PrEPi prescriber was the family practitioner (92.5%).

Conclusion: While the number of PrEPi initiations in primary care steadily increased over time, the profile of users remains similar to that observed before PrC initiation. The high rate of PrEPi not done by the patient’s family practitioner highlights potential barriers of sharing sexual health concerns with his own family practitioner. Tracking of biological tests required at PrEPi could be improved to conform compliance with national guidelines. Extending PrEPi to women and socio-economically disadvantaged people still requires raising awareness at target audiences and practitioners.

1142 Uptake of HIV prevention by Notified Seronegative Partners in HIV-Discordant Couples in Uganda
Edith Namulema1, Elizabeth Mutambuze1, Isaac Lwanga1, Allan Simwogerere1, Rachael Ankunda1, Tonny Tumwesigye1, Nelson Mugume2, Arthur G. Fitzmaurice3, Maria Baumbba1, ‘Mengo Hospital, Kampala, Uganda, 2Makere University College of Health Sciences, Kampala, Uganda, 3US Centers for Disease Control and Prevention Kampala, Kampala, Uganda

Background: Assisted Partner Notification (APN) is an effective HIV epidemic control strategy and is part of the interventions to increase case identification and reduce HIV transmission in Uganda. The focus for APN is un diagnosed contacts of index clients living with HIV. APN facilitates in linkage of sexual partners to HIV prevention (negative contacts in HIV status-discordant relationships) or treatment. However, failure of notified contacts to disclose their negative results to positive partners might expose them to ongoing HIV risk. Our objectives were to assess uptake and factors associated with HIV prevention among notified HIV-negative partners.

Methods: We analyzed cross-sectional data from partners of index clients tested January 2019–December 2022 at Mengo Hospital, Kampala, Uganda. After APN and testing seronegative at the hospital, we determined uptake of HIV retesting; disclosure of seronegative status to current sexual partner; reporting of abstinence, being faithful, condom use, and/or pre-exposure prophylaxis (PrEP); and safe male circumcision (SMC). We used multiple logistic regression (R4.22) to generate adjusted odds ratios and 95% confidence intervals (CI) of uptake of prevention methods.

Results: Among 3,068 elicited partners of 1,977 index clients, 89% (2,732) were notified; 61% (1,672/2,732) tested HIV-seronegative, 24% (657) positive, 12% (334) were in care, and 2.5% (69) declined testing. Of those testing negative, 93% (1,547) were reached for follow-up after 3-6 months; mean age was 32.1 years; 60% (929/1,547) were male. Only 44% (675) had retested. HIV status disclosure to seropositive partner was 24% (376); 86% (1,330) reported at least one prevention measure (SMC 35%), PrEP (5.8%), abstinence (17%), being faithful (33%), condoms (49%). Retesting was associated with age <20 (AOR=3.46; 95%CI 1.56-4.52), disclosure (AOR=8.83; 95%CI 6.54-11.9), and use of a prevention method (AOR=3.31; 95%CI 2.22-4.93). Partners reporting being faithful were 46% less likely to retest (AOR=0.54; 95%CI 0.41-0.70). Those using PrEP were three times more likely to disclose (AOR= 3.31; 95%CI 1.93-5.08). Ten (1.5%) partners seroconverted.

Conclusion: Low uptake of HIV prevention suggests ongoing HIV risk. Low retesting rates, such as among those who reported being faithful as a prevention method, suggest seronegative partners would not receive timely treatment. It is critical for HIV-seronegative partners to be actively followed up for disclosure, behavior change, and HIV prevention methods.

Lloyd B. Mulenga1, Sombo Fwoloshi2, Suliuni Sivile1, Linah Mwango2, Evelyn Mwamba3, Chofwe Chola1, Davies Kambamba4, Lottie Hachaambwa2, Aggry Mweemba1, C. William Wester5, Manji Pisingwa1, Prawlal Owoski2, Lamace Chirwa3, Cassidy W. Claassen4, 1University Teaching Hospital, Lusaka, Zambia, 2Cheb Lodge, Lusaka, Zambia, 3Vanderbilt University, Nashville, TN, USA, 4Maryland Global Initiatives Corporation, Lusaka, Zambia, 5University of Baltimore, Baltimore, MD, USA

Background: There is significant interest in HIV pre-exposure prophylaxis (PrEP) among women in sub-Saharan African countries like Zambia. Oral PrEP uptake with tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) may be limited by access to physicians to prescribe PrEP, and concern for kidney and bone mineral density abnormalities. PrEP with tenofovir alafenamide (TAF) and FTC has fewer bone and kidney side effects and thus may require less physician oversight and laboratory monitoring. We designed a 48-week trial to assess outcomes of a nurse-led PrEP service delivery model using TAF/FTC compared to standard-of-care (SOC) physician-led PrEP using TDF/FTC.

Methods: Quasi-experimental (pre-post) design study comparing TDF/FTC to TAF/FTC for oral PrEP among cis-gender women in six Ministry of Health facilities in Lusaka, Zambia. Inclusion criteria included HIV-negative, assigned female at birth, age 18 years of age, and eligible for PrEP per national guidelines. The control group was enrolled first and received SOC with physician-led oral TDF/FTC. The intervention group was enrolled thereafter and received nurse-led oral TAF/FTC. Study outcomes included uptake of PrEP, PrEP refill rates at 4 weeks, TVF metabolite concentrations at 24 and 48 weeks, PrEP persistence at 48 weeks, and HIV seroconversion.

Results: We screened 1,005 cis-gender women, of whom 900 (89.6%) were eligible for PrEP. 432 were enrolled in the SOC TDF/FTC group, and 450 were enrolled in the interventional TAF/FTC arm. PrEP refill rates at 1 month were 80% (347/432) in the TDF arm vs. 91.6% (412/450) in the TAF arm (p<0.001). Retention in PrEP services at 24 weeks were 35% (150/432) for the TDF arm vs. 77% (348/450) for the TAF arm (p<0.001). Participants in the intervention arm were 6 times more likely to continue PrEP at 24 weeks (odds ratio (OR)=6.36, p<0.001). At 24 weeks, TVF metabolite concentrations were ascertained in 106 (71%) of 150 individuals in the TDF arm compared to 280 (80.5%) of 348 in the TAF arm. HIV seroconversion rates at 24 weeks were identical in both study arms, with 1 (0.2%) of 432 participants in the SOC arm vs. 1 (0.2%) of 450 in the intervention arm.

Conclusion: In this non-randomized pre/post-trial, nurse-led PrEP with TAF/FTC showed significant advantages, including higher PrEP refill rates and improved PrEP persistence at 24 weeks compared to the physician-led SOC with TDF/FTC. HIV
1145 No Significant Interactions From Hormone Therapy on F/TAF-Based PrEP in Trans Men: iFACT Study

Akarin Hiransuthikul, Narukajorn Thammajaruk, Stephen Kerr, Rena Janamuyaaosok, Siriporn Nonenoy, Piranon Hongchoskait, Rapee Trichavaroji, Yardpiron Towon, Jakkrapatara Boonruang, Nipat Teeratakulpisarn, Tim R. Cresssey, Peter L. Anderson, Nittaya Phanuphak, for the iFACT Study Team

Chulalongkorn University, Bangkok, Thailand; Institute of HIV Research and Innovation (IHRI), Bangkok, Thailand; Chiang Mai University, Chiang Mai, Thailand; University of Colorado Anschutz Medical Campus, Aurora, CO, USA

**Background:** We previously observed comparable plasma and urine levels of tenofovir (TFV) and emtricitabine (FTC), as well as intracellular tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP) concentrations in peripheral mononuclear cells (PBMCs), when F/TAF-based oral daily PrEP was administered with and without feminizing hormone therapy (FHT) in trans women in Thailand. We assessed the potential impact of FHT on antiretroviral drug concentrations in the rectal compartment of trans women receiving oral daily F/TAF-based PrEP.

**Methods:** iFACT Study was a pharmacokinetic and safety study assessing the potential drug-drug interactions between FHT and oral daily F/TAF-based PrEP among trans women Thailand. Between January and February 2022, 20 trans women who had not undergone oophorectomy and had not received injectable FHT within the last 3 months were enrolled. Oral FHT (estradiol valerate 2 mg and cyproterone acetate 25 mg) was prescribed to participants at baseline until week 9, while oral daily PrEP (FTC 200 mg/TAF 25 mg) was initiated at week 3 until week 12. PK sampling was performed at week 3 (FHT without PrEP) and week 9 (FHT with PrEP) for estradiol; and weeks 9 (PrEP with FHT) and 12 (PrEP without FHT) for F/TAF PrEP drug parameters. Plasma, urine, and PBMCs samples were collected from all participants; and rectal tissue samples in subset.

**Results:** Ten participants who underwent rectal tissue sampling were included in this analysis. Median age and body mass index were 28.5 (IQR: 24-32) years and 21.2 (IQR: 19.9-21.9) kg/m², respectively. Median TFV-DP and FTC-TP concentrations in rectal tissue of weeks 9 and 12 were not statistically significant (TFV-DP, 37.6 [IQR: 21.4-45.8] vs 27.4 [IQR: 21.4-56.3] fmol/mg, p=0.19; and FTC-TP, 1.09 [IQR: 1.00-1.18] vs 1.09 [IQR: 1.00-1.18], p=0.17). Median pre-dose TFV-DP and FTC-TP concentrations were not significantly different between week 12 and 16 in PBMCs (C24 TFV-DP, 83.6 [72.0-102.3] vs 81.3 [63.1-100.8] fmol/10⁶ cells, p=0.72; and FTC-TP, 14.5 [12.4-17.0] vs 11.6 [8.0-14.5] fmol/mg, p=0.20). All participants had quantifiable TFV-DP and FTC-TP concentrations at both visits. Plasma concentrations in rectal tissue between weeks 9 and 12 were not statistically significant drug-drug interactions between FHT and oral daily F/TAF-based PrEP.

**Conclusion:** Intracellular TFV-DP and FTC-TP concentrations in rectal tissue were comparable when F/TAF-based PrEP was administered with and without FHT. These findings align with our previously data in the plasma, urine, and PBMCs compartments, indicating no clinically significant drug-drug interactions from FHT towards F/TAF-based oral daily PrEP are anticipated.

1146 Potential Drug Interactions From Hormone Therapy Toward F/TAF-Based PrEP in Trans Men: iFACT Study

Akarin Hiransuthikul, Narukajorn Thammajaruk, Stephen Kerr, Rena Janamuyaaosok, Siriporn Nonenoy, Piranon Hongchoskait, Rapee Trichavaroji, Yardpiron Towon, Jakkrapatara Boonruang, Nipat Teeratakulpisarn, Tim R. Cresssey, Peter L. Anderson, Nittaya Phanuphak, for the iFACT Study Team

Chulalongkorn University, Bangkok, Thailand; Institute of HIV Research and Innovation (IHRI), Bangkok, Thailand; Chiang Mai University, Chiang Mai, Thailand; University of Colorado Anschutz Medical Campus, Aurora, CO, USA

**Background:** We previously reported that plasma total testosterone concentrations were comparable in trans men receiving oral daily PrEP with menstruating hormone therapy (MHT). Herein, we report the impact of MHT on the PK parameters of oral daily F/TAF-based PrEP.

**Methods:** iFACT was a PK study evaluating the potential drug-drug interactions between MHT and oral daily PrEP among trans men Thailand. Ten participants who underwent rectal tissue sampling were included in this analysis. Median age and body mass index were 27.5 (22.5-33) years and 23.8 (20.5-25.2) kg/m², respectively. The geometric mean ratios (GMRs) (90%CI) of area under the concentration-time curve from 0 to 24 hours (AUC0-24) and maximum concentration (Cmax) were 0.97 (0.69-1.39) vs 1.00 (0.99-1.02), p=0.64; and 1.00 (0.99-1.02) vs 1.00 (0.99-1.02), p=0.51; and FTC-TP, 30.3 [5.1-143.0] vs 34.0 [29.0-153.8] fmol/mg, p=0.20.

**Conclusion:** Plasma levels of TFV and FTC, and intracellular concentrations of TFV-DP and FTC-TP were comparable when F/TAF-based PrEP was administered with and without MHT, suggesting no clinically significant DDIs from MHT towards F/TAF-based PrEP.

**Results:** Among 20 participants, median (interquartile range [IQR]) age and body mass index were 27.5 (22.5-33) years and 23.8 (20.5-25.2) kg/m², respectively. The geometric mean ratios (GMRs) (90%CI) of area under the concentration-time curve from 0 to 24 hours (AUC0-24) and maximum concentration (Cmax) were 0.97 (0.69-1.39) vs 1.00 (0.99-1.02), p=0.64; and 1.00 (0.99-1.02) vs 1.00 (0.99-1.02), p=0.51; and FTC-TP, 30.3 [5.1-143.0] vs 34.0 [29.0-153.8] fmol/mg, p=0.20.

**Conclusion:** Plasma levels of TFV and FTC, and intracellular concentrations of TFV-DP and FTC-TP in PBMCs, rectal and cervical tissue, were comparable when F/TAF-based PrEP was administered with and without MHT, suggesting no clinically significant DDIs from MHT towards F/TAF-based PrEP.

**Methods:** iFACT was a PK study evaluating the potential drug-drug interactions between MHT and oral PrEP among trans men Thailand. Ten participants who underwent rectal tissue sampling were included in this analysis. Median age and body mass index were 27.5 (22.5-33) years and 23.8 (20.5-25.2) kg/m², respectively. The geometric mean ratios (GMRs) (90%CI) of area under the concentration-time curve from 0 to 24 hours (AUC0-24) and maximum concentration (Cmax) were 0.97 (0.69-1.39) vs 1.00 (0.99-1.02), p=0.64; and 1.00 (0.99-1.02) vs 1.00 (0.99-1.02), p=0.51; and FTC-TP, 30.3 [5.1-143.0] vs 34.0 [29.0-153.8] fmol/mg, p=0.20.

**Conclusion:** Plasma levels of TFV and FTC, and intracellular concentrations of TFV-DP and FTC-TP were comparable when F/TAF-based PrEP was administered with and without MHT, suggesting no clinically significant DDIs from MHT towards F/TAF-based PrEP.
One-Year Declines in Bone Mineral Density Among Young Women Using TDF-Based PrEP and DMPA

Renee Heffron1, Timothy Muwonge2, Katherine K. Thomas3, Kidist Zewdie4, Timothy Ssebuliba5, Gabrielle Stein6, Susan Morrison7, Josephine Badarú8, Agnes Nakyamj9, Felix Bambizi9, Kenneth K. Muywana10, Christina Wyatt11, Michael T. Yin12, Flavia Kiweewa Matovu13, Andrew Mugagira14

1University of Alabama at Birmingham, Birmingham, AL, USA; 2Infectious Diseases Institute, Kampala, Uganda; 3University of Washington, Seattle, WA, USA; 4O’Heke University, Durban, KZ, USA; 5Columbia University, New York, NY, USA; 6Makerere University–Johns Hopkins University Research Collaboration, Kampala, Uganda

Background: Injectable depot medroxyprogesterone acetate (DMPA) is the most common contraceptive choice among young women in Uganda and nearby countries, where HIV burden is also high and HIV PrEP may be offered. DMPA and TDF-based PrEP have been associated with reductions in bone mineral density (BMD) when used consistently over multiple years. For young women who have not yet reached peak bone mass and choose to use DMPA, it is unknown whether concurrent PrEP use exacerbates declines in BMD.

Methods: We conducted a 2-year prospective observational study with women ages 16-25 years in Kampala, Uganda, who desired prevention for pregnancy and HIV. Women were provided either condoms or injectable DMPA for contraception and either condoms or FTC/TDF as oral PrEP for HIV prevention, according to their choices. Annual dual x-ray absorptiometry (DXA) scans were performed to measure BMD. We used tenefoivir-diphosphate (TDF-DP) quantification in dried blood spots and delivery of DMPA injections to determine exposure to each agent. Linear regression models estimated the difference in % BMD change from baseline to month 12 for women using oral PrEP and DMPA versus women using either agent individually or neither agent.

Results: Of 499 enrolled women with median age 20 years (IQR 18-21) and normal baseline distribution of BMD z-scores, discontinuation and re-starting of contraceptive and PrEP use was common during follow-up. Women using neither agent (n=39) experienced BMD growth of 2.0% at lumbar spine, 1.2% at hip, and 0.1% at femoral neck. Women with consistent use of both agents during pregnancy and HIV. Women were provided either condoms or injectable DMPA for contraception and either condoms or FTC/TDF as oral PrEP for HIV prevention, according to their choices. Annual dual x-ray absorptiometry (DXA) scans were performed to measure BMD. We used tenefoivir-diphosphate (TDF-DP) quantification in dried blood spots and delivery of DMPA injections to determine exposure to each agent. Linear regression models estimated the difference in % BMD change from baseline to month 12 for women using oral PrEP and DMPA versus women using either agent individually or neither agent.

Conclusion: We observed some significant difference in % BMD change of 1 year (n=22) experienced an average BMD loss of 1.04% in the lumbar spine at hip, 0.10% at femoral neck. Women with consistent use of both agents during pregnancy and HIV. Women were provided either condoms or injectable DMPA for contraception and either condoms or FTC/TDF as oral PrEP for HIV prevention, according to their choices. Annual dual x-ray absorptiometry (DXA) scans were performed to measure BMD. We used tenefoivir-diphosphate (TDF-DP) quantification in dried blood spots and delivery of DMPA injections to determine exposure to each agent. Linear regression models estimated the difference in % BMD change from baseline to month 12 for women using oral PrEP and DMPA versus women using either agent individually or neither agent.

Objective Assessment of Doxycycline PEP Use Among Cisgender Women in Kenya

Jenell Stewart1, Kevin Owuare2, Deborah Donnell3, Lauren R. Violette4, Josephine Odoyo5, Victor Omollo6, Felix Mogaka7, Matthew A. Spinelli8, Hideaki Okochi9, Monica Gandhi10, Elizabeth Bukusi11, Jared Baeten12, for the dPEP Kenya Study Team

1University of Minnesota, Minneapolis, MN, USA; 2Kenya Medical Research Institute, Kisumu, Kenya; 3Fred Hutchinson Cancer Center, Seattle, WA, USA; 4University of Washington, Seattle, WA, USA; 5University of California San Francisco, San Francisco, CA, USA

Background: Doxycycline postexposure prophylaxis taken following a condomless sexual exposure reduces incident bacterial STIs among men who have sex with men but not among cisgender women in initial trials. Adherence is a key component of effective biomedical interventions, and some initial trials of HIV PrEP among cisgender women reported null results due to low adherence, especially among those under 24 years old, despite having increased risk of HIV acquisition. Lack of efficacy reported in the dPEP Kenya Study were likely due to low use of doxycycline; factors associated with use have not yet been reported.

Methods: We conducted an analysis of doxycycline use among all follow-up visits of a randomly selected subset (n=50; 200 person-visits) of participants assigned to doxycycline PEP (doxycycline hydroclate 200mg taken within 72 hours of sex) within an open-label randomized trial among 449 women aged 18-30 years in Kisumu, Kenya. Participants returned quarterly over one year for STI testing and behavioral surveys; and provided hair samples for objective detection of doxycycline. The 1cm hair segment from the scalp, representing exposure over approximately the preceding month, was tested for doxycycline detectability (>0.020 ng/mg) using liquid chromatography-tandem mass spectrometry. Baseline and time-varying covariates potentially associated with doxycycline detection were assessed using modified Poisson regression with robust standard errors and generalized estimation equations.

Results: Doxycycline was detected in 29.0% (58/200) of hair samples, 32.6% (58/178) when censoring pregnancy time off doxycycline. Age of 24 years or older, an independent income source, more than one partner, and no primary sex partner were all associated with detection of doxycycline. After adjustment, older age and not having a primary partner both remain significantly associated with exposure. Common risk factors for STI exposure, e.g., higher frequency of sex, transactional sex, or prior STI, were not associated with detection of doxycycline. Participants reporting concern about getting an STI 34.4% (22/64) or concern that primary partner had other partners 29.1% (23/79) did not exhibit an increase in doxycycline detection.

Conclusion: Among young cisgender women taking HIV PrEP with a high prevalence and incidence of STIs, the use of doxycycline for STI prevention was overall low. Detection of doxycycline using objective measures was associated with older age and not having a primary sex partner. The figure, table, or graphic for this abstract has been removed.

Doxycycline Postexposure Prophylaxis to Prevent Trichomonas vaginalis Among Cisgender Women

Frederick Albertina Sesay1, Kevin Owuare2, Lauren R. Violette3, Deborah Donnell4, Josephine Odoyo5, Victor Omollo6, Felix Mogaka7, R. Scott McClelland8, Jennifer E. Balkus9, Elizabeth Bukusi10, Jared Baeten11, Jenell Stewart12, for the dPEP Kenya Study Team

1University of Washington, Seattle, WA, USA; 2Kenya Medical Research Institute, Kisumu, Kenya; 3Fred Hutchinson Cancer Center, Seattle, WA, USA; 4Kenya Medical Research Institute, Nairobi, Kenya; 5Hennepin Healthcare Research Institute, Minneapolis, MN, USA

Background: Trichomonas vaginalis, the most prevalent curable sexually transmitted infection (STI), disproportionately affects cisgender women, leading to reproductive complications and increased HIV acquisition risk. In-vitro studies suggest doxycycline as a potential treatment; however, no study has explored the ability of doxycycline to prevent T. vaginalis infection.

Methods: We conducted an open-label randomized trial among 449 women (18-30 years) taking oral HIV preexposure prophylaxis (PrEP) in Kisumu, Kenya. Participants were randomized to doxycycline postexposure prophylaxis (dPEP), 200mg within 72 hours of condomless sex, or standard of care (SOC), quarterly STI screening and treatment. All participants were followed for 12 months with quarterly visits for STI testing, including T. vaginalis testing (Cepheid GeneXpert) and data collection on various health and behavioral parameters. The trial had over 90% power to detect a 50% reduction in incident T. vaginalis infections. An intention-to-treat analysis utilizing generalized estimating equations was performed.

Results: Baseline characteristics were similar in the dPEP (n=224) and SOC groups (n=225). Overall, participants had a median age of 24 years, reported a

Table: Estimated difference in the percentage change in bone mineral density (BMD) from baseline to month 12 for women using oral PrEP and DMPA versus women using either agent individually or neither agent.

<table>
<thead>
<tr>
<th>Source of Bone Density</th>
<th>Group</th>
<th>n</th>
<th>Mean</th>
<th>Variance</th>
<th>Standard Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine (BMD)</td>
<td>DMPA</td>
<td>22</td>
<td>-2.15%</td>
<td>1.12%</td>
<td>0.003</td>
<td>-2.29% - 2.01%</td>
</tr>
<tr>
<td></td>
<td>PrEP</td>
<td>22</td>
<td>-2.14%</td>
<td>1.23%</td>
<td>0.003</td>
<td>-2.28% - 2.00%</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>39</td>
<td>1.87%</td>
<td>0.11%</td>
<td>0.007</td>
<td>-2.16% - 1.22%</td>
</tr>
</tbody>
</table>

Note: DMPA, depot medroxyprogesterone acetate; PrEP, preexposure prophylaxis; BMD, bone mineral density.
Bacterial STI Trends Associated With the October 2022 Doxy-Prophylaxis Recommendation, San Francisco

Andy Liu, Jiayuan Hao, Trevor A. Pickering, Jeffrey D. Klausner
University of Southern California, Los Angeles, CA, USA

Background: Sexually transmitted infections (STIs) have been on the rise in the United States over the past decade and disproportionately impact men who have sex with men and transgender women. The San Francisco Department of Public Health (SFDPH) issued guidance in October 2022 recommending clinicians to prescribe doxy-prophylaxis as an STI prevention strategy to cis men and trans women who report condomless sex with a cis male or trans female partner and have had a bacterial STI in the past year. We assessed the trends of male rectal chlamydia, male rectal gonorrhea, and adult male syphilis cases to explore the association between the doxy-prophylaxis policy and bacterial STI rates among males in San Francisco.

Methods: We reviewed publicly available monthly STI reports on male rectal chlamydia, male rectal gonorrhea, and adult male syphilis among MSM and TGW receiving PrEP at an STI clinic. The difference was calculated for each STI. To account for temporal trends related to COVID-19, we included 2 breakpoints, November 2021 (period of Omicron) and November 2022 (period of policy introduction). We compared the slopes of the fitted regression lines before and after. Two-sided P<0.05 was considered statistically significant.

Results:

- **Male Rectal Chlamydia:**
  - Before policy: 11,200 (79.0%)
  - After policy: 9,130 (59.0%)
  - Difference: 2,070 (8.0%)

- **Male Rectal Gonorrhea:**
  - Before policy: 12,200 (30.0%)
  - After policy: 3,100 (18.0%)
  - Difference: 9,100 (24.0%)

- **Adult Male Syphilis:**
  - Before policy: 6,200 (51.0%)
  - After policy: 3,100 (29.0%)
  - Difference: 3,100 (24.0%)

Conclusion: The doxy-prophylaxis recommendation was associated with a significant decrease in male rectal chlamydia and continued decline in male rectal gonorrhea and syphilis. Given the ecologic nature of the study, further confirmation is needed.

Table 1. Incident T. vaginalis by Study quarter

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Doxyprophylaxis PEP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>225</td>
<td>224</td>
</tr>
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1151 Doxy-PEP Effectiveness in Men Who Have Sex With Men (MSM) and Transgender Women (TGW) on HIV PrEP

Oliver Bacon, Robert P. Kahn, David V. Glidden, Madeleine Sanikanan, Monica Levy, Trang Q. Nguyen, Stephanie E. Cohen
San Francisco Department of Public Health, San Francisco, CA, USA, University of California San Francisco, San Francisco, CA, USA

Background: Doxycycline post-exposure prophylaxis (doxy-PEP) reduced the risk of chlamydia (CT), gonorrhea (GC) and early syphilis (ES) in MSM and TGW in randomized trials. Little is known about doxy-PEP effectiveness on STI positivity outside of clinical trials.

Methods: Starting 11/3/22, doxy-PEP was offered to patients receiving PrEP at San Francisco City Clinic who were eligible according to citywide guidelines. We assessed visit-level STI positivity at all visits among MSM and TGW PrEP patients who had at least one clinic visit pre (11/3/21-11/2/22) and post (11/3/22-11/2/23) guideline release. STI positivity was defined for GC and CT as the proportion of visits with a GC or CT test with at least one positive result, and for ES as the proportion of visits with a syphilis test that identified a new ES case. For those who ever initiated (i.e., were prescribed or self-reported use of doxy-PEP users), we compared STI positivity at visits pre and post doxy-PEP initiation. For those who did not initiate doxy-PEP (non-users), we compared STI positivity at visits pre and post 11/1/22. We compared the reduction in positivity for users vs. non-users using a generalized estimating equation model with robust variance to account for repeated measures. All data were routinely collected during clinical visits.

Results: 506 patients had visits in both periods: 367 users and 139 non-users.
- Positivity decreased for CT (RR 0.10, 95% CI 0.05–0.21) significantly in users but not in non-users (RR 0.73, 95% CI 0.44–1.21) (Table); the decrease in positivity was significantly greater among users (p<0.001). ES positivity decreased significantly (RR 0.44, 95% CI 0.21–0.92) in users but not non-users (RR 0.68, 95% CI 0.20–2.30) (Table); these decreases were not significantly different (p=0.56). GC positivity decreased non-significantly for both groups (Table).

Conclusion: Doxy-PEP significantly reduced positivity for CT and early syphilis among MSM and TGW receiving PrEP at an STI clinic. The difference was significantly greater than the reduction in CT seen in doxy-PEP non-users. There was no significant reduction in GC in doxy-PEP users or non-users. The reasons for reduction in CT positivity and new early syphilis cases in non-users is unclear and warrants further investigation.
Early Adopters: Implementation of Doxycycline Postexposure Prophylaxis in a Boston Health Center

Kenneth H. Mayer1, Michael Traeger1, Sy Gitin, Jessica Kraft1, Taimur Khan1
1Fenway Health, Boston, MA, USA, 2Bumet Institute, Melbourne, VIC, Australia

Background: The use of 200 mg of doxycycline within 72 hours after condomless sex (“doxyPEP”) has been shown to decrease the incidence of bacterial sexually transmitted infections (bSTI) in 2 randomized trials that enrolled cisgender men who have sex with men (MSM) and transgender women. However, limited data are available to inform the implementation of this novel STI prevention intervention in primary care settings. This report describes the doxyPEP roll-out in a Boston health center that specializes in primary care for gender and sexual minorities.

Methods: Data from the clinic’s electronic medical records were abstracted. Among all patients screened for a bSTI in 2023, bivariate logistic regression was used to compare characteristics of those receiving doxyPEP and those not receiving doxyPEP. DoxyPEP uptake (proportion receiving doxyPEP in 2023) among those with an active PrEP prescription in 2022-2023, PLHIV, and those tested for, and those diagnosed with, a bSTI in 2022 were also calculated.

Results: Between January 26 and September 6, 2023, 1120 patients received a doxyPEP prescription. By March, monthly doxyPEP starts exceeded 100, with most doxyPEP patients being White (73.3%), non-Latinx (81.7%), and cisgender MSM (92.2%), with a mean age of 39.1 years. Among all patients screened for a bSTI in 2023 (N=4976), those receiving doxyPEP were more likely to be PrEP users (73.8% vs. 39.9%, odds ratio (OR)=4.25, 95%CI=3.67-4.93), have private insurance (85.4% vs. 71.3%, OR=2.37 (1.96-2.81)), have been diagnosed with a bSTI in 2022 (31.3% vs. 15.0%, OR=1.67 (1.42-1.98)), and less likely to be people living with HIV (PLHIV: 8.6% vs. 18.1%, OR=0.42 (0.34-0.53)) than those who did not receive doxyPEP. DoxyPEP users did not differ from non-users by age, race or ethnicity. DoxyPEP uptake was 24.1% (827/3427) among patients with an active PrEP prescription in 2022-2023, PLHIV, and those tested for, and those diagnosed with, a bSTI in 2022 were also calculated.

Conclusion: Interest in STI prophylaxis is nearly universal and use is already common among GBM engaged in online networks. Some are using medications and dosing that are untested as STI prophylaxis. Findings can inform doxyPEP implementation strategies, including education on effective dosing and monitoring of actual use.

Impact of Doxycycline as STI PEP on the Gut Microbiome and Antimicrobial Resistance Gene Expression

Victoria T. Chu1, Abigail Glasscock1, Deborah Donnelly1, Cole Grabow1, Ryan Ward1, Christina Love1, Stephanie E. Cohen1, Julia C. Dombrowski1, Connie Celum1, Annie Luukemeyers1, Charles Langeller1
1University of California San Francisco, San Francisco, CA, USA, 2Channing Laboratory, Harvard Medical School, Boston, MA, USA, 3University of Washington, Seattle, WA, USA, 4Fred Hutchinson Cancer Research Center, Seattle, WA, USA, 5University of Alabama at Birmingham, Birmingham, AL, USA

Background: The DoxyPEP trial compared doxy-PEP use (DP) to standard of care (SOC) for 501 MSM/TW living with HIV or on HIV pre-exposure prophylaxis (PrEP). The impact on the gut bacterial microbiome and its associated antimicrobial resistance genes (ARGs) is unknown.

Methods: The DoxyPEP trial randomly assigned doxy-PEP users (DP) to standard of care (SOC) for 501 MSM/TW living with HIV or on PrEP. Metagenomic sequencing of DNA and RNA (DNA-seq, RNA-seq) was performed on self-collected rectal swabs from enrollment (MO) and month 6 (M6) from 50 SOC and 100 DP participants matched by HIV infection status. We analyzed all samples passing minimum sequencing quality control standards. From DNA-seq data, we compared bacterial microbiome diversity and total bacterial abundance in 59 DP and 30 SOC participants, and assessed baseline tetracycline (TO) and ARG abundance at enrollment. We then compared changes in ARG expression at MO and M6 using RNA-seq data of samples from 46 DP and 24 SOC participants. Bacterial abundance was normalized per million reads sequenced (reads per million,
Background: Syphilis has had a resurgence in the United States. Untreated syphilis infections have detrimental health effects, and improved screening procedures are needed to control the epidemic. We performed a retrospective, deidentified cross-sectional analysis of individuals attending the Johns Hopkins Emergency Department (JHHED) to determine the prevalence of syphilis and HIV, as well as the socio-demographic characteristics associated with presumed active infection (PAI) of syphilis.

Methods: Remnant serum samples from 1951 unique patients attending the JHHED in Baltimore, between January and February 2022 were collected. Demographic variables were extracted from the electronic medical record, and personal identifiers were removed prior to sample testing. Testing was performed with the CAPTIA Syphilis T-Palladium, Sure-Vue RPR, and Serodia TP-PA treponemal and non-treponemal assays. PAI was classified by positive treponemal serology or a RPR non-treponemal titer ≥ 1:8. "Not Presumed Active Infection" (NPAI) was classified by positive treponemal serology, and no high RPR titer. "True Negative" patients had a nonreactive initial serology. Patient HIV status was determined with the BioRad HIV 1+2 O ELISA and the BioRad Genius HIV 1+2 assays. Fisher's exact and Wilcoxon rank-sum tests were performed to determine the sociodemographic factors associated with PAI.

Results: Among 1951 samples tested, 1.2% (23/1951) had PAI, 4.1% (80/1951) had NPAI, and 4.5% (87/1951) were living with HIV. Of the 103 treponemal positive samples, 17.4% (18/103) were living with HIV. Prevalence of positive treponemal serology was higher in men (6.7%) than women (3.0%), and was differential by race (Hispanic 7.8%, non-Hispanic black 6.9%, non-Hispanic white 2.2%). Patients with PAI were significantly younger than those without (median: 35 [IQR: 29–48]; median: 47 [IQR: 32,62], p=0.019). Six of nine women with PAI were in childbearing age. Though PAI was higher among people living with HIV than those not (5.8% vs 1.0%, respectively, p=0.003), the majority of PAI 78.3% (18/23) were found in HIV-negative people. In comparison to those who reported having a primary care provider (PCP), patients who did not had 5.7-times higher odds of having PAI (OR, 5.7 [95% CI: 2.1-15.5]).

Conclusion: One in 20 ED patients had positive treponemal serology for syphilis and several were also living with HIV. Updated screening protocols to include populations most recently infected are imperative to mitigate the resurgence of syphilis nationally.

Jarisch-Herxheimer Reaction in Patients With Syphilis With or Without Prior Antibiotic Prophylaxis

Samuel Lazzarin, Andrea Poloni, Giorgia Carrozzo, Giacomo Pozza, Chiara Fusetti, Francesco Caruso, Serena Reato, Maddalena Matone, Andrea Giacomelli, Maria Vittoria Cosu, Andrea Gori, Giuliano Rizzardini, Spinello Antonini, Davide Moschese

Luigi Sacco Hospital, Milan, Italy

Background: Jarisch-Herxheimer reaction (JHR) is a transient clinical phenomenon that may occur within 24 hours after penicillin treatment in individuals with syphilis infection. JHR incidence ranges from 9 to 31%, reaching 56% in early syphilis when proactively investigated. Aim of our study is to determine the incidence of proactively investigated JHR in individuals with or without prior antibiotic prophylaxis.

Methods: We enrolled consecutive patients diagnosed with syphilis from April to September 2023 undergoing penicillin treatment. Twenty-four hours after receiving the first penicillin dose, a phone call was made by a healthcare professional to assess symptoms referable to JHR and the exposure to antibiotics (active against Treponema pallidum) before penicillin administration. JHR was defined, after excluding other possible causes, by the presence of at least one of: fever, chills, new or worsening rash, headache and myalgias. Individuals were categorized as exposed or not to antibiotics before penicillin if they received at least a 48-hours course of antibiotics in the 7 days before the first penicillin dose.

Results: Ninety-five individuals were enrolled, with a median age of 41 years (IQR 30–50), including 93 (98%) males and 78 (82%) people living with HIV, of whom 61 (78%) with an HIV-RNA <50 cp/mL. Seventy patients (74%) were MSM, 14 (15%) transgender women and 11 (11%) heterosexuals. The
distribution of syphilis stages was: 5% primary, 12% secondary, 41% early latent, 41% late latent and 1% neurosyphilis. Previous penicillin treatment was reported in 50 patients (53%). Median RPR titer at diagnosis was 1.8 (IQR 1.2-1.16). We identified 71 patients (75%) with a study-defined antibiotic exposure: 67 (94%) amoxicillin and 4 (6%) doxycycline. The median cumulative dose of amoxicillin was 9.5 g (IQR 6-12 g) and doxycycline 1200 mg (IQR 950-1400 mg). Six patients (incidence 6.3% (95% CI 2.3-13.7%)) developed JHR, with fever being the most common clinical manifestation (4 in the antibiotic exposed and 2 in the antibiotic unexposed group). Among the subset of 55 patients with early syphilis (58%), 4 (incidence 7.3% (95% CI 1.1-10.2%)) had JHR (2 in the antibiotic exposed and 2 in the antibiotic unexposed group).

**Conclusion:** The observed overall low incidence of JHR, even lower in the early syphilis group (which accounted for a higher proportion of cases when compared to previous studies), could be attributable to antibiotic prophylaxis, although a relatively low RPR titer might also play a role.

### 1158 Increasing Syphilis Prevalence Among MSM Across India Despite Improvements in the HIV Care Continuum
Matthew M. Hamill\(^1\), Mihili P. Gunaratne\(^2\), Allison M. McFall\(^1\), Hussain Syed Iqbal\(^2\), Canjeeravaram K. Vasudevan\(^1\), Santhanan Anand\(^1\), Sunil Suhas Solomon\(^1\), Shrutii H. Mehta\(^1\), Aylur K. Srikrishnan\(^1\), David O. Celentano\(^2\), Gregory M. Lucas\(^1\)
\(^1\)The Johns Hopkins University School of Medicine, Baltimore, MD, USA, \(^2\)The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, \(^1\)YR Gaitonde Center for AIDS Research and Education, Chennai, India

**Background:** Syphilis rates have increased in men who sex with men (MSM) in high income countries. Infectious syphilis is associated with HIV acquisition in MSM. Nationally representative syphilis prevalence data from MSM in low- and middle-income settings are lacking. We evaluated changes over 5 years in syphilis and high titer (HT) RPR prevalence across 10 Indian cities.

**Methods:** As part of a cluster-randomized trial to evaluate integrated HIV prevention, testing, and treatment services, respondent-driven sampling (RDS) accrued samples of MSM (n=1000/city) in 10 Indian cities between 2012-2013 and again in 2016-2017. Participants underwent serological testing for syphilis (RPR). Additional testing was performed on RPR-positive stored sera to determine titers (high ≥ 1:8, low <1:8). The integrated services intervention did not have a statistically significant effect on syphilis prevalence. We evaluated associations with HT prevalence from 2016–17 using RDS-weighted multivariable multilevel multinomial regression models stratified by HIV status.

**Results:** 9984 and 9991 MSM were sampled in 2012-2013 and 2016-2017, respectively. During this time, HIV prevalence increased from 7.4% to 15.3% with improvements across the HIV care continuum (33.4% vs 59.1% viral suppression). At the same time, syphilis prevalence increased in all cities; the median prevalence increase from 2012 to 2017 was 4.7% (range: 1.6 - 7.5%) with increase in HT (2.7% [1.2 - 4.8%]) accounting for more of the overall increase than low titer (1.6% [1.3 - 2.2%]) (Fig 1). Of 9991 MSM with lab confirmed syphilis, only 3.2% reported a prior diagnosis. Among MSM living with HIV, significant correlates of HT (vs. negative) included being older, HIV viral load >1000 copies/ml, and reporting running out of money in the prior 12 months. Among MSM without HIV, correlates of HT included being older, having prior gonorrhea or chlamydia diagnoses and any STI symptom. Regardless of HIV status, kothi identity (prefer receptive anal sex) and recent condom use with male partners was associated with higher HT prevalence.

**Conclusion:** Syphilis prevalence increased within all 10 cities in 5-years. Associations with HT suggests targeted interventions like STI-PEP are needed. Of concern, HT was associated with viremia in men living with HIV suggesting a greater likelihood of HIV transmission to sex partners not on HIV PrEP. Augmented adherence support and early syphilis treatment is required.

Alex de Voux\(^1\), Wellington Maruma\(^2\), Maboe Morifi\(^3\), Modiehi Maduma\(^2\), Joy Ebonwu\(^1\), Sithembile Dlamini Nqeteko\(^1\), Landon Myer\(^2\), Tendesayi Kuta\(^2\)
\(^1\)University of Cape Town, Cape Town, South Africa, \(^2\)National Institute for Communicable Diseases, Johannesburg, South Africa, \(^3\)World Health Organization, Geneva, Switzerland

**Background:** Congenital syphilis (CS)-vertical transmission of syphilis during pregnancy-can lead to adverse fetal outcomes including stillbirth and neonatal death. In South Africa (SA), CS is notifiable, however, notifications have been low leading to concerns of underreporting. In addition, infant data accompanying CS surveillance reports (CNFs) are minimal limiting evaluation of missed prevention opportunities. Since January 2022, CNFs were supplemented with CS-specific case investigation forms (CIFs) with additional maternal and infant clinical history. We reviewed CS CNFs and CIFs reported in SA from January 2020–June 2022 to identify gaps in the CS prevention cascade.

**Methods:** We reviewed CNFs prospectively collected during January 2020–June 2022 and matched them to submitted CIFs to review infant and maternal clinical history and determine the proportion of pregnant women (PW) (1) enrolled in antenatal care (ANC), (2) tested for syphilis during pregnancy, and (3) treated for syphilis, amongst those diagnosed, ≥28 days before delivery.

**Results:** During January 2020–June 2022 930 CNFs and 667 CIFs were submitted. Record linkage provided a final dataset of 343 matched CNF-CIF pairs, 37% (343/938) of total CNFs. Among 343 matched CNF-CIF pairs, 56% (n=195) of pregnant women (PW) had ≥1 ANC visit documented, 87% (n=298) were tested for syphilis and of these 88% (n=261) tested positive and 70% (n=210) were treated. Of those tested during pregnancy, 40% (n=120) were tested ≥28 days before delivery. Limiting to PW with ≥1 ANC visit documented (n=195), 98% (n=191) were tested, and of these 86% (n=164) tested positive and 78% (n=152) were treated. Overall, 28% of treated PW received the first dose ≥28 days before delivery, while 18% of PW treated <28 days before delivery tested positive for syphilis ≥28 days before delivery.

**Conclusion:** Total SA CS cases reported during January 2020–June 2022 translated to a crude CS rate (36 per 100,000 live births) half the CS rate estimated by the World Health Organization (86 per 100,000 live births) for SA in 2022. Evaluation of the CS prevention cascade relied on supplemental infant and maternal information and was limited to less than half of total cases reported. PW with early engagement in ANC had improved syphilis testing and treatment outcomes, but testing earlier in pregnancy did not always guarantee timely treatment highlighting need to identify barriers to timely treatment following a positive syphilis test during pregnancy.

### 1160 Routine Emergency Department Screening Increases Syphilis Diagnosis Among Pregnant Patients
Kimberly A. Stanford, Eleanor Friedman, Joseph Mason, Aniruddha Hazra, John Schneider
University of Chicago, Chicago, IL, USA

**Background:** Considering the recent surge in congenital syphilis, novel means of reaching vulnerable populations for testing and treatment are needed. The CDC recently suggested screening outside traditional prenatal care settings might be an effective strategy. As the primary source of healthcare for many communities with limited access to care, visits to the emergency department (ED) may represent a crucial opportunity for syphilis detection and congenital syphilis prevention.

**Methods:** A routine, opt-out, syphilis screening program for all ED patients under age 65 was implemented in the ED of a large, urban, tertiary care hospital
in Chicago. Prior to that, testing occurred at clinician discretion following the standard of care. This study retrospectively reviewed all ED encounters among pregnant people for the two-year periods before and after implementation of the screening program. Syphilis cases were defined by a combination of positive serology, rapid plasma regain (RPR) titers, and clinical history derived from chart review. Descriptive statistics were used to evaluate changes in screening and diagnosis rates, as well as demographic and clinical trends.

**Results:** A total of 9,165 ED encounters involving pregnant patients were identified. In the two years before the intervention, 296 of 4,764 (6.2%) encounters included testing for syphilis, which increased almost eight-fold after the intervention, to 2,307 of 4,401 (52.4%) encounters. There were 3 (1.1% of screened population) syphilis cases identified before the intervention, which quintupled to 16 (0.7%) after the intervention. Screened patients were predominantly non-Hispanic Black (94.3% before, 92.1% after) and had public insurance (72.3% before, 72.5% after), reflecting local demographics. Notably, of all pregnant patients diagnosed with syphilis through the screening program, only 5 (31.2%) were tested for other sexually transmitted infections (STIs), 7 (43.8%) presented to the ED with abdominal or pelvic pain, and none presented with symptoms of an STI.

**Conclusion:** This study found that a non-targeted screening program dramatically increased syphilis screening and diagnosis rates among pregnant patients, the majority of whom did not present with concern for STI. Implementing routine ED syphilis screening in high prevalence communities will be key to addressing the syphilis epidemic, eradicating congenital syphilis, and addressing major health care disparities.

1161 **Syphilis Screening and Incident Infection Rates in HIV Treatment and HIV PrEP Programs in BC, Canada**

**Junine Toy,1 Paul Sereda,1 Raquel M. Espinoza2, Erin Ready3, Viviane Dias Lima4, Kate Salters5, Peter Phillips4, Rolando Barrios1, Julio Montaner1**

1 British Columbia Centre for Excellence on HIV/AIDS, Vancouver, Canada; 2 St Paul’s Hospital, Vancouver, Canada

**Background:** In Canada, syphilis continues to affect gay, bisexual and other men who have sex with men (gbMSM), and increasingly heterosexual persons and youth. We characterize syphilis testing and incidence rates among British Columbia (BC) HIV PrEP and HIV Drug Treatment Program (DTP) clients.

**Methods:** Adults ≥18 years enrolled in BC PrEP and DTP with program contact (lab test, drug dispensed) between 1-Jan-2018 to 31-Dec-2022 were included (followed to 30-Jun-2023). Demographics, HIV risk group, syphilis testing rates (per person-year (PY)), using a reverse-sequence algorithm with treponemal EIA, and incidence rates (per 100PY) were estimated.

**Results:** Overall, 20,033 clients [median (Q1-Q3) age 44 (33-57) years; 90% cis-men, 9% cis-women, 1% trans-women, 0.3% trans-men, 0.3% other genders were included (PrEP (n=10,422), DTP (n=9,557), and PrEP/DTP (n=54), the latter including clients switching from PrEP to DTP after HIV infection). 95% of cases had ≥1 syphilis test (PrEP (n=10,240), DTP (n=8645) and PrEP/DTP (n=54)), for which the testing rates were 3.40, 2.75, and 3.13 per PY, and incidence rates were 5.03, 3.78, and 14.73 cases per 100PY, respectively. See Figure. Among PrEP clients, those reporting prior bacterial rectal STI or syphilis as their PrEP eligibility criteria, had the highest incident syphilis rate (10.84 per 100PY), followed by those with baseline HIRI-MSM score ≥10 plus ≥1 HIV risk factor (8.44 per 100PY). High syphilis rate (8.19 per 100PY) among clients with baseline HIRI score ≥25 was observed. Youth (under 30), and those aged 30-39 had higher syphilis rates in DTP than in PrEP (8.45 and 7.09 per 100PY, respectively) vs. (4.98 and 5.36 per 100PY, respectively), gbMSM in the DTP had similar syphilis rate to the PrEP program overall (5.68 vs. 5.03 per 100PY), as well as similar screening rates (3.15 vs. 3.40 per 100PY). For heterosexuals and persons who have ever injected drugs in DTP, syphilis rates were 1.33 and 1.13 per 100PY, respectively. For cis-women with ≥1 syphilis test (n=1470/1748, 84%), testing rate was 1.97 per PY and incidence rate 0.96 per 100PY across programs.

**Conclusion:** High syphilis rates were observed in PrEP clients reporting baseline STI, multiple HIV risk factors, and HIRI-MSM score ≥25. This was similar for gbMSM and younger persons living with HIV. Notably, PrEP clients who subsequently acquired HIV had very high incident syphilis rates. These findings will help inform future public health interventions, such as doxYP EP

1162 **Altered Cellular Immune Response in the Context of Syphilis and Acute HIV Coinfection**

Jasper Mundt, Lennart Nicksch, Carola Horn, Ute Sanderadura de Silva, Isabelle Suarez, Max Augustin, Clara Lehmann

Cologne University Hospital, Cologne, Germany

**Background:** The co-infection of syphilis and acute HIV infection is gaining clinical significance due to rising co-infection rates and the unique interaction between these two sexually transmitted infections. Syphilis enhances HIV transmission and acquisition, while HIV accelerates the progression of syphilis. This study aimed to investigate the cellular immune responses in the context of acute HIV/syphilis co-infection.

**Methods:** A cross-sectional analysis was conducted, comparing four cohorts of 15 patients each (total N=60): acute HIV infection with syphilis (aHIVS), acute HIV infection without syphilis (aHIV), chronically coinfected syphilis+/HIV+ patients on antiretroviral treatment (aHIVS), and healthy controls (CTRL). Flow cytometry assessed PD-1, HLA-DR, and CD38 expression on various T-cell subsets (CD3+CD4+, CD3+CD8+, T cells (CD8)), memory T cells (CD4+CD45RO+ memory T-cells (TM), CCR7±CD27± memory subsets (TCM: T central memory, TEM: T effector memory, TTM: Transitional memory), antigen-presenting cells (CD4+CD45RO– TN), regulatory T-cells (CD49b+LAG-3+ (TR1)), and plasmacytoid dendritic cells (BDCA2+CD123+ (pDC)). IFN gamma (IFNγ) levels were measured in plasma using Simoa technology (Quanterix).

**Results:** In patients with acute HIV/syphilis co-infection, the frequency of TCD4 (p<0.0001) and TCD8 (p<0.0001) cells was significantly lower compared to other groups, while CD38 expression was highest (p<0.0001). The frequency of pDC (p<0.0001) and TR1 (p=0.0365) decreased significantly in aHIVS, aHIV, and chIVS5 groups compared to CTRL. Interestingly, T central memory cells were significantly increased in aHIVS (p=0.0005). Notably, the highest PD-1 expression on type 1 regulatory T-cells was observed in acutely HIV-coinfected patients (p=0.0003), and it correlated positively with viral plasma load (r=0.6 p=0.0314). Furthermore, the highest levels of IFNγ were found in acutely HIV-coinfected patients.

**Conclusion:** The impaired T-cell function, activation, and exhaustion observed in acute HIV/syphilis coinfection result in reduced control of HIV replication. Longitudinal studies of patients coinfected with HIV and syphilis, especially after initiating antiretroviral treatment, are warranted to investigate their effects on the HIV reservoir.

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1163 **Gaps in Sexual and Reproductive Health Care Among Cisgender Women With Diagnosed HIV**

Sharoda Dasgupta, Stacy Crim, John Weiser, Angela Blackwell, Jen-Feng Lu, Margaret Lampke, Ada Dieke, Robyn N. Fanfair

Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** Cisgender women with HIV (WWH) are defined by more than their HIV viral loads. Comprehensive women’s health, including sexual and reproductive health (SRH), is an important component of overall health and well-being. We used data from the CDC’s Medical Monitoring Project (MMP) to describe nationally representative estimates of SRH outcomes among WWH of reproductive age.

**Methods:** During 6/2018–5/2021, MMP interviews were conducted and medical records were abstracted to ascertain data on SRH among WWH. Among WWH aged 18–44 years (N=855), we reported weighted percentages and calculated prevalence differences (PDs) with predicted marginal means and
accompanying 95% confidence intervals (CIs) to quantify differences between groups.

**Results:** Overall, 86.4% of WWH reported receiving a cervical Pap smear in the past 3 years. Of sexually active WWH, 38.3% had documented sexually transmitted infection (STI) testing for syphilis, gonorrhea, and chlamydia in the past 12 months, per CDC guidelines. Of WWH who engaged in vaginal sex, 88.9% used ≥1 form of contraception in the past 12 months, including 16.3% who used long-acting reversible contraceptive methods (Figure). Over half (53.4%) had ≥1 pregnancy since their HIV diagnosis, of whom 81.5% had ≥1 unplanned pregnancy, 24.6% had ≥1 miscarriage or stillbirth, and 9.8% had ≥1 abortion. WWH living in households <100% of the federal poverty level (FPL) were less likely to receive Pap smears (PD: -9.13; 95% CI: -14.77 – -3.50) and more likely to have unplanned pregnancies (PD: 16.57; 95% CI: 2.63 – 30.50) than those living in households ≥139% of the FPL. However, WWH attending Ryan White HIV/AIDS Program (RWAP)-funded clinics were more likely to receive STI testing at their HIV care facility than those who did not attend RWAP-funded facilities (PD: 20.08; 95% CI: 10.55 – 29.61). In MMP states that did not expand Medicaid, WWH with Medicaid coverage were less likely to have Pap smears (PD: -12.39; 95% CI: -20.49 – -4.29) and more likely to have unplanned pregnancies (PD: 27.17; 95% CI: 11.53 – 43.19)* than those with private coverage. *Should be interpreted with caution due to small sample sizes.

**Conclusion:** Many WWH have suboptimal SRH screening and pregnancy outcomes, particularly those living in poverty. Expansion of safety net programs that provide substantial coverage of SRH services, including Medicaid, RWAP, and Title X, could help improve care access and outcomes among WWH.

### 1164 Genital and Extragynal Sexually Transmitted Infections Among Reproductive Age Women in Southern US

**Nicholas F. Nogueira,1 Paola Beato Fernandez,2 Yue Pan,1 Ana Salazar,1 Maria G. Rodriguez,1 Gray Kelsey,1 Patricia Del Carmen Raccamarich,2 Daniel Westreich,3 Seble Kassaye,1 Elizabeth F. Topper,1 Aida Rana1, Deborah Konkle-Parker,4 Deborah Jones Weiss,4 Anandi N. Sheth,1 Maria L. Alcaide1**

1University of Miami, Miami, FL, USA, 2University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 3Gynecology, Washington, DC, USA, 4The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 5University of Alabama at Birmingham, Birmingham, AL, USA, 6University of Mississippi Medical Center, Jackson, MS, USA, 7Emory University, Atlanta, GA, USA

**Background:** Sexually transmitted infections (STIs) are highly prevalent among women of reproductive age (WRA) and increase the risk of HIV acquisition and transmission. However, the burden of genital and extragynal STIs is understudied among WRA in the U.S. Estimates of disease are urgently needed, including among women with and without HIV, to inform sex-specific screening guidelines.

**Methods:** Cross-sectional data from 519 cisgender women, 18-45 years old enrolled in the STAR cohort from March 2021 to January 2023 at 6 Southern US sites were analyzed. Women with and without HIV (at least one HIV risk behavior in the previous 5 years). Socio-demographic and behavioral assessments were performed using structured interviewer-administered questionnaires. Nucleic-Acid Amplification Test (NAAT) was performed regardless of symptoms on self-collected urine, rectal, and pharyngeal swabs to detect chlamydia, gonorrhea, and trichomiasis. Socio-demographic characteristics, risk factors, and rates of STIs were tabulated and group comparisons by HIV status were examined.

**Results:** Mean age was 34.6 ± 6.95 years; 79.6% were Black, 15.5% White, and 11.4% Hispanic. Overall, 52.8% had never married, 45.7% had a monthly income of $1500 or less, and 36.0% completed high school. Women had a median of 1 (IQR 1–2) male sexual partners in past year, 44.1% reported condomless vaginal sex in the past year, and 49% reported a prior STI: 35.2% reported a lifetime history of chlamydia, 26.0% gonorrhea, and 30.6% trichomiasis. Current STI detected by NAAT did not differ by HIV status (22.0% HIV+ vs. 20.5% HIV−; p=0.946), vaginal chlamydia (2.1% HIV+ vs. 1.5% HIV−; p=0.915), rectal chlamydia (2.7% HIV+ vs. 3.1% HIV−; p=0.568), pharyngeal chlamydia (1.3% HIV+ vs. 0.0% HIV−; p=0.43), vaginal gonorrhea (3.8% HIV+ vs. 2.3% HIV−; p=0.277), rectal gonorrhea (0.9% HIV+ vs. 1.6% HIV−; p=0.843), pharyngeal gonorrhea (1.3% HIV+ vs. 0.8% HIV−; p=0.832), and trichomiasis (14.3% HIV+ vs. 13.0% HIV−; p=0.937).

**Conclusion:** Prevalence of genital and extragynal chlamydia and gonorrhea, and genital trichomiasis are high among WRA with and without HIV infection. The implications for women’s reproductive health and HIV transmission highlight the importance of extragynal STI testing for women with HIV or vulnerable to HIV infection.

### 1165 Prevalence and Risk Factors Associated With HPV Infections Among Women with HIV in Meru, Kenya

**Celestine K. Nyamari,1 Anthony Kebira2, Frank Onyambu3**

1Centre for Molecular Biosciences and Genomics, Nairobi, Kenya, 2Kenyatta University, Nairobi, Kenya, 3Meru University of Science and Technology, Meru, Kenya

**Background:** Cervical cancer, caused by Human Papilloma Virus (HPV) is the leading cause of preventable deaths among women. High incidence and high mortality for cervical cancer are reported in low- and middle-income countries where immunocompromised HIV-infected women exhibit an increased risk. We determined circulating high-risk HPV genotypes in HIV-infected women in Meru, Kenya and identified risk factors associated with HPV infections in a cross-sectional study of 273 women aged 25 to 64 years.

**Methods:** Sociodemographic and clinical details were collected using a questionnaire. Cervical specimens were obtained using a self-sampling technique, followed by HPV DNA extraction and real-time PCR targeting 24 high-risk genotypes with differentiation of HPV 16, 18, and 45. Descriptive statistics were used to summarize baseline characteristics, while logistic regression analysis was utilized to determine the risk factors associated with HPV infection.

**Results:** Out of the 273 tested samples, 60.81% (N=166) tested positive for high-risk HPV broken down as HPV 18 (37.73 %), HPV 45 (32.32 %), other high-risk HPV types (14.29 %), and HPV 16 (12.45 %). The prevalence of multiple infections with HPV 16 and 18 was 8.42% (N=23). We further found 54.82% (N=90) had undergone Pap smear and 45.18% (n=75) had undergone VIA/VILI within the last six months with 4 individuals reporting abnormal results. Notably, among the 161 participants who reported normal results in their Pap smear/VIA tests, HPV positivity was detected. In our tentative analysis we used logistic regression to show the risk factors for high risk HPV were age of 33 to 44 years (OR: 0.45, 95% CI [0.206-0.991], p=0.045), and contraceptive use (OR: 0.496, 95% CI [0.247-0.996], p=0.047).

**Conclusion:** Our study reveals a concerning high prevalence of high-risk HPV in Kenya and identifies two significant risk factors among HIV-infected women in Meru. Women aged 35–44 years exhibit an increased risk for HPV, while contraceptive use is another risk factor. This underscores the urgent need for tailored interventions and enhanced screening strategies among HIV-infected women in Meru, Kenya. The identified risk factors highlight areas where proactive measures can make a significant impact on reducing cervical cancer risk. Our findings contribute to the growing evidence on HPV burden in Kenya, reinforcing the call for effective public health measures to prevent cervical cancer.

### 1166 One-Dose HPV Vaccine Durability in a Moderate HIV Prevalence Setting: Mathematical Modeling Analyses

**Christine L. Hathaway,1 Grace Umutesi,2 Jesse A. Heitner1, Rachel Jackson1, Christine Wangeci4, Wesley Mugambi5, Lydia Khalayi4, Valerian Mwenzida6, Lynda M. Olouch2, Mary Nyangasi1, Rose E. Jalingo7, Nelly R. Mugo8, Lydiah Khalayi4, Jesse A. Heitner3**

1Massachusetts General Hospital, Boston, MA, USA, 2University of Washington, Seattle, WA, USA, 3State University of New York Upstate Medical University, Syracuse, NY, USA, 4National Vaccination and Immunization Program, Nairobi, Kenya, 5National Cancer Control Program, Nairobi, Kenya, 6Kenyatta University, Nairobi, Kenya, 7Kenyatta University, Nairobi, Kenya, 8National Cancer Control Program, Nairobi, Kenya

**Background:** The World Health Organization recommends a 1-dose human papillomavirus (HPV) vaccination strategy to increase coverage and accelerate cervical cancer elimination. However, the duration of efficacy of 1-dose is uncertain among women living with HIV (WLHV); immune dysregulation may
limit vaccine durability. We modelled the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya. We examined a 1-dose HPV vaccination strategy assuming a 30-year efficacy period (EP) and a 10-year waning period (WP), with lifelong coverage. We aimed to evaluate the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya.

Methods: We developed a validated dynamic compartmental transmission model for Kenya, simulating the impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes. We examined the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya. We examined the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya. We examined the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya. We examined the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya.

Results: The 1-dose HPV vaccination strategy could significantly reduce cervical cancer incidence and mortality. The 1-dose HPV vaccination strategy could significantly reduce cervical cancer incidence and mortality. The 1-dose HPV vaccination strategy could significantly reduce cervical cancer incidence and mortality. The 1-dose HPV vaccination strategy could significantly reduce cervical cancer incidence and mortality.


1168 Genital Immune Correlates of Prevalent and Incident Herpes Simplex Virus 2 (HSV-2) Infection
Sujai Udayakumar, Jane Flanagan, Sanja Huibner, Mary Kinga, Rhoda Kabutu, Eratus Irungu, Pauline Nyagare, Peter Muthoga, Wendy Adhiambo, Helen Weiss, Janet Seeley, Tara Beatley, Joshua Kimani, Rupert Kaul

Background: This longitudinal study was nested within the Maisha Fiti cohort of 1003 females who sell sex in Nairobi, Kenya. Among 731 HIV-negative participants, HSV-2 serostatus was assessed by Kalon HSV-2 IgG assay at baseline and follow up. Soluble genital immune factors were assayed in cervicovaginal specimens using a multiplex electro-chemiluminescent immunoassay (MSD), and socio-behavioural characteristics assessed by questionnaire. Socio-behavioural characteristics and immune parameters were compared between HSV-2 seropositive and seronegative participants using chi-square and Mann-Whitney U tests and examined using linear regression models controlling for potential confounders. Socio-behavioural characteristics and immune parameters were compared between HSV-2 seropositive and seronegative participants using chi-square and Mann-Whitney U tests and examined using linear regression models controlling for potential confounders. Socio-behavioural characteristics and immune parameters were compared between HSV-2 seropositive and seronegative participants using chi-square and Mann-Whitney U tests and examined using linear regression models controlling for potential confounders. Socio-behavioural characteristics and immune parameters were compared between HSV-2 seropositive and seronegative participants using chi-square and Mann-Whitney U tests and examined using linear regression models controlling for potential confounders.

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Results: Of 11921 clients attending ANC, 953 (8.0%) were approached for integration testing and treatment in this study demonstrate acceptability and feasibility. However, less than 10% of pregnant clients were approached, owing to limited testing capacity. Further implementation research is urgently needed to address scalability, in order to expand integrated STI testing at ANC clinics.

Kevin Martin1, Chido Oziwa Chikwari2, Ethel Dauya1, Estella Mukuka3, Joseph D. Tucker4

Background: There is limited testing for CT, NG, TV, or HBV available across public sector health services, including ANC, in Southern Africa. There is urgent need to implement strategies for prevention, detection, and treatment for these highly prevalent STIs. The high uptake and provision of same-day results and treatment in this study demonstrate acceptability and feasibility. However, less than 10% of pregnant clients were approached, owing to limited testing capacity. Further implementation research is urgently needed to address scalability, in order to expand integrated STI testing at ANC clinics.

Methods: We examined a dynamic compartmental transmission model for Kenya, simulating the impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes. We examined the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya. We examined the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya. We examined the potential impact of a 1-dose HPV vaccination strategy on cervical cancer outcomes for WLHIV in Kenya.

Results: If Kenya achieves UNAIDS targets by 2030 without additional HPV vaccination, we expect a 1.7% cervical cancer incidence reduction amongst WLHIV compared to a baseline of no vaccination and current ART coverage. Achieving UNAIDS targets as well as 90% coverage of a 1-dose HPV vaccine for WLHIV would further reduce incidence to 85.1%. A 1-dose scenario with lifelong efficacy would have similar reductions in incidence and mortality. The worst-case waning scenario of 15-year EP/10-year WP would weaken the incidence reduction by a difference of 17% compared to the 1-dose lifelong efficacy scenario, while the best-case waning scenario of 30-year EP/20-year WP would limit the reduction by 0.1%. Catch-up vaccination has the potential to avert some of the impact of HPV vaccine waning.

Conclusion: Assuming 1-dose efficacy results are consistent for women living with and without HIV, durability differences between dosing strategies will not significantly impact cervical cancer outcomes for WLHIV. While waning vaccine efficacy for 1-dose could increase cervical cancer cases compared to multi-dose strategies, general population vaccination could mitigate some of this change. Empiric data on single-dose HPV vaccine efficacy and duration for women living with HIV are needed.
1169 HIV and STIs in US Bisexual Men and Gay Men: Clinical Implications and Service Needs

Thomas Carpino,1 Kaitlyn Atkinson,1 Cristian S. Acero,1 Ishaq Lucas,1 Hector Moran,1 Sarah Murray,1 Travis H. Sanchez,2 Stefano Barali

1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2Emory University, Atlanta, GA, USA

Background: Bisexual individuals comprise over half of LGBTQ+ persons in the United States (US), yet remain understudied in HIV and STI research. Goals to end the US HIV epidemic require reaching bisexual cisgender men who have sex with men (MSM) who may have different sexual health behaviors and prevention/ care needs than gay-identified MSM.

Methods: We analyzed data from self-identified bisexual (n=778) and gay (n=3290) MSM who participated in the 2022-2023 American Men’s Internet Survey (AMIS), a cross-sectional online study of MSM aged 15 and older in the US who had same-sex anal sex within the past 12 months. We evaluated self-reported HIV and STI diagnosis (chlamydia, gonorrhea, syphilis, and mpxo) within a past year, testing activities, sexual practices, PrEP use, experiences of outness, and healthcare-related stigma, and associations with sexual identity.

Results: Lifetime HIV testing was 9.1% less prevalent in bisexual men compared to gay men (p=0.001), and 58.7% (n=451) of bisexual men received an HIV test in the prior year (Table). Lifetime PrEP use was 18.6% lower in bisexual compared to gay men (p=0.001). Among MSM who had initiated PrEP, 73.1% of bisexual men (n=182) reported PrEP use in the past 12 months, compared to 78.0% (n=1235) of gay men. Bisexual men reported lower rates of past-year STI testing (p=0.001) and diagnosis (chlamydia, mpxo: p=0.01; syphilis: p=0.016). Compared to gay men, bisexual men were 32.5% less likely to be out to providers (p=0.001) and 12.5% less likely to discuss sex with providers (p=0.001). Among bisexual men, outness to healthcare providers was not associated with anticipated healthcare stigma, but among gay men, outness was associated with lower anticipated healthcare stigma (p=0.001).

Conclusion: Despite lower HIV and STI prevalence among bisexual men in our study, we found disparities in uptake of PrEP, suggesting a need for research into barriers to PrEP and or tailored messaging for this group. Greater anticipated healthcare stigma, lower outness, and less frequent conversations about sexual practices among bisexual men highlight a need to ensure healthcare providers adequately address the HIV and sexual health needs of this population. These findings provide a more comprehensive understanding of considerations to inform testing, prevention, and reducing anticipated healthcare stigma among bisexual men.

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1170 Relevance of Asymptomatic STIs in a High-Risk Population of MSM Undergoing Periodic Screening

Rosario Palacios,2 Cristina Gómez-Ayerbe,2 María López-Jódar,1 Isabel A. Pérez-Hernández,1 Isabel Vicedo,1 María Villalobos,1 Victoria García,1 Jesús Santos Hernández1

1Vich de la Victoria, Málaga, Spain

Background: One of the limitations in the control of STIs is that many of them are asymptomatic, making diagnosis, treatment, and contact tracing challenging. The objective of this study is to analyze the significance of asymptomatic STIs in a high-risk population (MSM) that undergoes regular screening for these infections.

Methods: This was a single-center study involving two populations: our cohort of MSM on PrEP (Pre-Exposure Prophylaxis) and People Living with HIV (PLWHV) followed up at our clinic, both at risk of STIs, who undergo STI screening every 3-6 months (including serological tests, cultures, and nucleic acid amplification tests in pharyngeal, rectal, and urethral/urine samples). The following STIs were analyzed: Neisseria gonorrhoeae (NG), Chlamydia trachomatis (CT), Lymphogranuloma venereum (LGV), and syphilis. Study period: November 1, 2021, to June 30, 2023. Proportions were compared using the chi-squared test or Fisher’s exact test. Statistical analysis was performed using SPSS 24.0 software.

Results: During this period, STI screening was conducted on 788 individuals on PrEP and 456 PLWHV, all MSM. A total of 560 STIs were diagnosed (359 individuals on PrEP and 201 in PLWHV; p=0.755); NG 194 (34.6%), syphilis T30 (23.2%), CT 121 (21.6%), and LGV 79 (14.1%) and 36 (6.4%) were other STIs; 416 (74.2%) were asymptomatic. Table 1 provides details of symptomatic and asymptomatic STIs in both groups. Some subjects had NG and/or CT in multiple sites. Coincident STIs were detected in 20.3% of the cases. There were no seroconversions among individuals on PrEP.

Conclusion: Asymptomatic STIs are strikingly prevalent in this high-risk MSM population. No differences in clinical presentation were observed between individuals on PrEP and PLWHV for any of the studied STIs. There is an urgent need to implement new prevention strategies to control STIs in these high-risk populations.

<table>
<thead>
<tr>
<th>STI</th>
<th>PrEP (n=788)</th>
<th>PLWHV (n=456)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Symptomatic</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Pharyngeal/NG</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>CT</td>
<td>122 (15.6%)</td>
<td>0</td>
</tr>
<tr>
<td>LGV</td>
<td>79 (10.2%)</td>
<td>0</td>
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</tbody>
</table>

Some subjects had NG and/or CT in multiple sites. Table 1: STI included in PrEP and PLWHV. N (%)
1172 Low Prevalence of Sexually Transmitted Infections Among Adolescents and Young Adults Living With HIV

Lynda M. Glooche, Paul Mwangi, Jane Gacheru, Irene Njeru, Kenneth Ngure, Linda Eckert, Denise A. Galloway, Anna Wald, Ruamun V. Barnabas, Nelly R. Mugul

Kenya Medical Research Institute, Nairobi, Kenya, 1Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, 2University of Washington, Seattle, WA, USA, 3Fred Hutchinson Cancer Center, Seattle, WA, USA, 4Massachusetts General Hospital, Boston, MA, USA

Background: Approximately 50% of sexually transmitted infections (STIs) are concentrated in people within the age range of 15 to 24 years. This coincides with age estimates at sexual debut. This cohort is of particular public health concern due to the potential consequences of STI transmission to new sexual partners. In a longitudinal cohort study of young females and males living with HIV, we assessed the prevalence of Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infections.

Methods: We enrolled 158 participants who had previously received the quadrivalent HPV vaccine and collected genital samples for CT and NG infections at enrolment and again at month 12. Nucleic acid amplification testing (NAAT) was performed on genital swabs to detect NG and CT, using the Gen-Probe APTIMA test. Cross-sectional data on demographic, medical and sexual history were gathered at enrolment. Descriptive statistics were employed to establish the baseline demographic characteristics. STI diagnoses were correlated with the age at sexual debut, gender, partner’s age at first sexual activity, education level, financial dependency and viral load.

Results: At enrolment 158 participants provided samples and 155 at month 12 visit. At enrolment 9/158 (5.7%) were diagnosed with CT or NG and at month 12, 16/155 (10.3%). Two participants had a positive result at both time points. Among those diagnosed with STIs at 12-month visit, 12/16 (75.0%) were females, 13/16 (81.3%) had never married and 9/16 (56.3%) were financially dependent. HIV RNA viral load (VL) ≥ 1000 was noted among 3/16 (26.6%) of those diagnosed with an STI, while more than 50% of participants with VL ≤ 1000 had STIs, 12/16(75%). In a multivariable logistic model, STI and older age reduced odds by 0.56 times per year (aOR 0.56, 95% CI 0.34-0.85). Tertiary education lowered odds by 0.03 times versus primary education (aOR 0.03, 95% CI 0.04-3.30). Substance use in the past six months increased the odds by 6.75 times (aOR 6.75, 95% CI 1.29-46.84). Having an older first sexual partner increased odds by 1.25 times (aOR 1.25 (1.06-1.50), while abstaining at younger age reduced odds by 0.65 times (aOR 0.65, 0.46-0.90).

Conclusion: Young men and women living with HIV had lower STI prevalence than other comparable population level age cohorts. Higher education and delayed sexual debut were protective while older sexual partner, female gender and financial dependency had increased risk, emphasizing the need for structural social protection interventions.

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1173 Reaching ECP Users With HIV and PEP Services in Kenyan Pharmacies

Nicholas Kipkurui, Dorothy Oketch, Scholastica Wanjiru, Gerald O. Owuor, Alloys Kolon, Elizabeth Bukusi, Alison Roxby, Kawango Agot

Impact Research and Development Organization, Kisumu, Kenya, 1University of Washington, Seattle, WA, USA

Background: Adolescent girls and young women (AGYW) seeking emergency contraceptive pills (ECP) to prevent conception also face increased risk of HIV acquisition due to implied unprotected sex. AGYW who are eligible for ECP are therefore likely to also be eligible for post-exposure prophylaxis (PEP) to reduce their risk of HIV acquisition. Pharmacies, where most AGYW purchase ECP, can serve as convenient outreach points for HIV prevention services including PEP. We assessed the willingness of AGYW seeking ECP to test for HIV and initiate PEP

Methods: We assessed willingness to test for HIV and initiate PEP as the main outcomes in a study among AGYW aged 15-24 seeking ECP in 5 pharmacies in Kisumu and Nairobi, Kenya, between May and August 2023. We trained pharmacy providers on research ethics, documentation, screening and consenting. They collected data via REDCap at baseline, with follow-up conducted within 10 days post enrollment. Baseline data captured socio-demographics, use of ECP, HIV risk perception, HIV testing history, willingness to test for HIV and willingness to initiate PEP. Post survey data captured their experience in seeking HIV testing and PEP. Participants were issued an information sheet on HIV testing and PEP and those interested referred to nearby health facilities for services. Data were summarized using descriptive statistics.

Results: We screened 297 AGYW and enrolled 200; mean age was 22 years; 90.0% were single; 10.0%, 41.7% and 48.2% had primary, secondary, and post-secondary education, respectively. 41.5% perceived themselves to be at high HIV risk and 28.5% at medium risk; 28.5% felt at low-risk despite seeking ECP. Table 1 shows those willing to test for HIV and to initiate PEP compared to those who actually went for testing after being educated on why they would be eligible. Overall, 50.7% AGYW took up testing compared to 95.5% who were willing. The most preferred testing modality was self-testing but since this was unavailable, most tested at the pharmacy; 25% tested at government facilities. Of those who tested, 80.2% tested within 3 days of enrolment. The 49.3% who did not test/take PEP cited low perceived risk (20.3%), fear of knowing HIV status (10.8%), awareness of partner’s status (9.5%), distance (6.8%), and procrastination (20.3%) as barriers.

Conclusion: The findings underscore the potential of pharmacies to enhance access to HIV testing and PEP initiation among at-risk AGYW by providing convenient outreach points.

1174 Stability of Penile Bacteria Associated With HIV Serocconversion, Inflammation, and Cells (BASICS)


1University of Washington, Seattle, WA, USA, 4University of Toronto, Toronto, Canada, 3Medical Research Council, London, UK, 5Massachusetts General Hospital, Boston, MA, USA

Background: Studies have defined specific penile anaerobic bacteria associated with HIV serocconversion, Inflammation and Cells (BASICS) in uncircumcised men. However, little is known regarding the colonization dynamics of BASICS in the coronal sulcus, or if they colonize other male genital tract sites outside of the coronal sulcus.

Methods: Swabs from 97 uncircumcised men were collected at the coronal sulcus, outer foreskin, penis shaft, and distal urethra in a cross-sectional study in Rakai, Uganda; 47 participants were then sampled longitudinally over 8 weeks. We characterized the penile microbiome and determined three key BASICS (Prevotella bivia, Peptostreptococcus anarobius, and Dialister micraerophilus) prevalence, proportional and absolute abundance by 16S rRNA qPCR and V3V4 amplicon sequencing. We compared overall microbiome composition by PERMANOVA and BASICS abundances between sites by pair-wise Mann-Whitney Test. We also compared stability of BASICS in the coronal sulcus to other penile commensal bacteria using residence and return times, which measure the time between disappearance and subsequent re-emergence of a taxon, respectively. We imputed abundance thresholds associated with increased HIV serocconversion risk for P. bivia, P. anarobius, and D. micraerophilus using published data (Prodger et al., 2021) to characterize dynamics of BASICS abundance over the 8-week study.

Results: Overall microbiome composition differed significantly across penile sites, with BASICS most prevalent and abundant in the coronal sulcus as compared to other sites. In most study participants, P. bivia, P. anarobius, and D. micraerophilus were detected at least once over the 8-week study period (62%, 70%, 64%, respectively), but fewer participants were colonized at all visits (17%, 17%, and 9% respectively) (Fig. 1A). All BASICS had shorter residence times in the coronal sulcus than Corynebacterium, a ubiquitous skin-associated
integrating HIV/STI prevention services into gender-affirming care programs for susceptibility. Our findings add to the scant body of knowledge available on the biomarkers is a potential underlying mechanism that can affect HIV/STI

Conclusion: Income, hormonal contraceptive use, and sexual behavior were similar between the MHT and non-MHT groups (GraphPad Prism 10.0.2). Age, race, lower levels of antimicrobial biomarkers Elafin (p<0.001), SLPI (p=0.0085), and TNFα (p=0.0053), and MIP1α (p<0.0001) were significantly elevated levels of inflammatory biomarkers Myeloperoxidase (p=0.0101), TNFα (p=0.0053), and MIP1α (p<0.0001). The MHT group also had significantly increased levels of inflammatory biomarkers Myeloperoxidase (p=0.0101), TNFα (p=0.0053), and MIP1α (p<0.0001) compared to the control group. Age, race, income, hormonal contraceptive use, and sexual behavior were similar between the groups.

Methods: We recruited 72 AFAB participants from the Washington, DC metro area (aged 18–44, sexually active, HIV-negative) and conducted a cross-sectional study comparing 36 TMNB participants who had been on MHT for at least six months with a control group of 36 cisgender/non-binary participants not on MHT. Participants provided clinical and demographic data and self-collected vaginal swabs. The concentrations of a panel of inflammatory and antimicrobial immune biomarkers in samples were assessed by ELISA. Statistical analyses were performed using Mann-Whitney U tests to compare biomarker values between the MHT and non-MHT groups.

Results: Compared to non-MHT controls, the MHT group had significantly elevated levels of inflammatory biomarkers Myeloperoxidase (p=0.0101), TNFα (p=0.0053), and MIP1α (p<0.0001). The MHT group also had significantly higher levels of antimicrobial biomarkers Elafin (p<0.001), SLPI (p=0.0085), and Human Beta-Defensin-2 (p=0.0033) compared to the control group. Age, race, income, hormonal contraceptive use, and sexual behavior were similar between the groups.

Conclusion: We found evidence of vaginal immune dysregulation in TMNB individuals receiving MHT. Dysregulation of inflammatory and antimicrobial biomarkers is a potential underlying mechanism that can affect HIV/STI susceptibility. Our findings add to the scant body of knowledge available on the immunomodulatory effects of synthetic testosterone in the vagina and may inform future studies on sexual health in TMNB. Our findings do not negate MHT as a clinically safe standard of care in TMNB, but point to an indication for integrating HIV/STI prevention services into gender-affirming care programs for individuals on MHT.
the greatest individual impact: averaged across all MSAs, HIV incidence was projected to fall by 49% if PrEP were scaled up to 25% of the eligible city population, and by 39% if retention in HIV care were increased to 95%. Some cities (such as Los Angeles) benefited more from improvements in PrEP coverage, while others (such as Baltimore) benefited more from increased retention. The impact of expanding interventions to different populations also varied. In Atlanta, 51% of the maximum added benefit of the combined intervention came from targeting only young Black and Hispanic MSM. By contrast, in San Francisco, 64% of the maximum added benefit resulted from expansion from this group to include all MSM and PWID.

Conclusion: This analysis provides information to local decision-makers as they seek to identify the combinations of interventions and risk groups that will maximize impact with limited resources in their cities, ultimately helping chart a strategic roadmap to the US HIV policy through 2035.

1178 Evolving Trends in Early ART Initiation in South Africa: An Analysis of Integrated HIV Program Data

Dorina Onoya1, Khumbo Shumba1, Cornelius Ntetary1, Dickman Gareta2, Evelyn Lauren3, William MacLeod4, Koleka Mlisana5, Jacob Bar6, Matthew P. Fox7
1Health Economics and Epidemiology Research Office, Johannesburg, South Africa, 2Africa Health Research Institute, Mbabane, South Africa, 3Boston University, Boston, MA, USA, 4National Health Laboratory Service, Johannesburg, South Africa

Background: South Africa (SA) has progressively improved HIV treatment guidelines to ensure rapid and sustained viral suppression. We describe the trends of the time between HIV diagnosis and the initiation of antiretroviral therapy (ART) for patients entering HIV care between 2010 and 2017.

Methods: We conducted a prospective cohort study, utilizing integrated data from the clinic-based Three Integrated Electronic Registers (TIERs.net) and the National Health Laboratory Service (NHLS) databases across four SA provinces (KwaZulu Natal, Mpumalanga, Limpopo and North West). The study population consisted of individuals diagnosed with HIV, entering in care between January 2010 and September 2017. Entry into care date was defined as either the first CD4 date from the NHLS data or the HIV diagnosis date from the TIER data. The time from entry to ART initiation (date of ART start noted in TIER data) was classified as same-day (HIV diagnosis date), 2-6 days, 7-89 days, and ≥90 days after entry into care. A trend analysis of the number of patients and proportions initiated by these subgroups was compared over time.

Results: Among the 1,319,239 individuals with linked NHLS and TIER data, entering care within the study period, 1,316,410 had started ART. Most were female (69.5%), with a median age of 31 years (Interquartile Range (IQR): 24-39). The number of patients starting ART decreased over time but the median CD4 at entry increased from 298 cells/μl (IQR: 171-463) in 2010 to 321 cells/μl (IQR: 173-500) in 2017. In the early stages of the epidemic, the majority of HIV patients initiated ≥90 days after entering HIV care, but by the end of the study period this was less than 10.0%. The percentage of patients starting ART ≥90 days after diagnosis decreased from 57.3% for 2010-2011 to 11.4% in 2016-2017. Conversely, same-day ART increased from 17.4% (2010-2011) to 36.0% (2016-2017). The percentage of patients initiated between 2-6 days post-diagnosis showed an upward trend, from 1.2% in 2010-2011 to 12.0% in 2016-2017. Additionally, the percentage of patients initiated within 7 to 89 days varied, starting at 24.1% in 2010-2011, increasing to 42.6% in 2012-2013 but then decreased to 12.0% in 2016-2017. Consistent trends were observed across provinces.

Conclusion: Our results reveal evolving trends in rapid ART initiation in South Africa, underscoring the importance of ongoing efforts to ensure timely ART initiation for all individuals living with HIV.

1180 Ending the HIV Epidemic in Atlanta: A Mixed-Methods Study to Support the Local HIV/AIDS Response

Micah Piske1, Bohdan Nosyk1, Justin C. Smith1, Bianca Yeung1, Benjamin Ennis2, Xiao Zang1, Patrick S. Sullivan3, Wendy S. Armstrong1, Melanie Thompson1, Gaea Daniel1, Carlos del Rio2
1University of British Columbia, Vancouver, Canada, 2Simon Fraser University, Burnaby, Canada, 3Positive Impact Health Centers, Atlanta, GA, USA, 4University of Minnesota, Minneapolis, MN, USA, 5Emory University, Atlanta, GA, USA

Background: Four counties within the Atlanta, Georgia 20-county eligible metropolitan area (EMA) are currently prioritized by the US ‘Ending the HIV Epidemic’ (EHE) initiative which aims for 90% reduction in HIV incidence by 2030. Disparities driving Atlanta’s HIV epidemic warrant an examination of local epidemiology, service availability, and organizational capacity to reach...
EHE targets. We conducted a mixed-methods evaluation of the Atlanta EMA to assess geographic HIV epidemiology and distribution of services, service needs, and organization infrastructure to implement or expand services for each pillar of the EHE initiative.

**Methods:** We collected 2021 county-level data from multiple sources including: AIDSvu (HIV prevalence and new diagnoses), the Centers for Disease Control and Prevention web-based tools (HIV testing and pre-exposure [PrEP] locations), and the Georgia Department of Public Health (HIV testing, PrEP screenings, viral suppression, and partner service interviews). We additionally distributed an online survey to key local stakeholders working at major HIV care agencies across the EMA to assess availability of services, unmet needs, and organization infrastructure during June to December 2022. The Organizational Readiness for Implementing Change (ORIC) questionnaire assessed organization climate for services in need of scale-up or implementation.

**Results:** We found racial/ethnic and geographic disparities in HIV disease burden and service availability across the EMA - particularly for HIV testing and PrEP in the EMA’s southern counties. Five counties not currently prioritized by EHE (Clayton, Douglas, Henry, Newton, and Rockdale) accounted for 16% of the EMA’s new diagnoses, but <9% of its 177 testing sites and <7% of its 130 PrEP sites. Survey respondents (N=48; 52% care providers, 42% other health agency staff, 10% people living with HIV) reported high unmet need for HIV self-testing kits, mobile clinic testing, HIV case management, peer outreach and navigation, integrated care, housing support, and transportation services. Respondents highlighted insufficient existing staffing and infrastructure to facilitate the necessary expansion of services, and the need to reduce inequities and address intersectional stigma.

**Conclusion:** Service delivery across all EHE pillars must substantially expand to reach national goals for metro Atlanta. High-resolution geographic data on HIV epidemiology and service delivery with community input can inform equitable strategies for local EHE efforts.

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**Figure:** Atlanta Eligible Metropolitan Areas (AMA) – Rates of people living with HIV per 100,000 county population in 2021 and facilities with HIV testing and PrEP prevalent within the AMA. Epidemic surveillance and sites with HIV testing, pre-exposure prophylaxis [PrEP] and syringe service programs (SSPs) are displayed.

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1182 Strategic Advice and Expert Procurement Accelerates the Optimization to DTG in the Americas

Omar M. Sued, Nora Giron, Kemel Hallar, Monica Alonso, Ruben Mayorga, Christopher Lim
Pan American Health Organization, Washington, DC, USA

**Background:** The WHO guidelines recommend dolutegravir (DTG) as the preferred anchor drug for first-line ART, as for second-line ART after a NNRTI failure. DTG has a higher resistance barrier and efficacy, fewer side effects, and a safer profile than other options. The transition to DTG increases viral suppression and ART durability. The Strategic Fund (SF) of the Pan American Health Organization (PAHO) is a technical cooperation mechanism for pooled procurement of essential medicines and health supplies that meet international standards. PAHO provides technical assistance to countries on rational selection and use of medicines and health technologies, demand planning, and strengthening of supply management systems. Here we describe our experience supporting countries to access DTG for adults and children.

**Methods:** Quantitative, descriptive analysis of purchase orders from Latin American and Caribbean countries between January 2018 to September 2023. All products containing DTG were included in the analysis. For comparison, we included all NNRTIs, PIs, and other integrase inhibitors, but excluded NRTI drugs to avoid duplication. We calculated the average annual cost of treatment per unit procured, weighted by the number of units procured. Correlations were calculated using the Pearson coefficient (r).

**Results:** Adult DTG-based treatments procurement increased from 13% in 2018 (58,135 annual treatments) to 81% in 2023 (240,282 annual treatments procured up to September). Non-DTG treatments decreased from over 390,000 treatments in 2016 to 97,000 in 2022 and 57,880 by September 2023. In addition, the annual unit prices of DTG-based treatments decreased by 40% since 2018 (from 69 USD to 41.45 USD a year), while non-DTG regimens increased by 15% in the same period (from 75.72 USD to 86.76 USD a year). The acquisition of treatment was inversely associated with the cost (r=-0.925). For pediatrics, dispersible pDTG 10mg was introduced in 2021 and continued to increase from 16% to 54% by September 2023. The use of pDTG represented a 90% price reduction compared to other regimens (53 USD a year compared to 549 USD a year).

**Conclusion:** SF and PAHO technical cooperation increased the access to generic DTG-based ART for adults and children in Latin America and the Caribbean. The transition to DTG represented significant savings due to the lower price of DTG.
1183 Evaluating Barriers to Care Among Adults With HIV Who Are Virally-Unsuppressed in Philadelphia

Ngwi Tayong, Tanner Nassau, Kathleen Brady
Philadelphia Department of Public Health, Philadelphia, PA, USA

Background: Barriers to HIV care lead to decreased access to and engagement in care resulting in lower rates of viral suppression, and in turn delays in progress towards Ending the HIV Epidemic (EHE). We sought to assess the proportion of virally unsuppressed individuals whose detectable viral load can be attributed to identified barriers of HIV care.

Methods: We used weighted data from the 2015-2021 cycles of the Medical Monitoring Project (MMP) among residents of Philadelphia, Pennsylvania. Weighted frequencies for barriers to care were calculated overall and by viral suppression status. We used generalized linear regression models to calculate the prevalence ratios for each barrier, adjusted for age and gender. We calculated the population attributable fraction (PAF) for the proportion of virally detectable PWH who could have become virally suppressed had they not experienced that specific barrier to HIV care. All relative risks and PAFs were stratified by race/ethnicity.

Results: There were an estimated 7,541 individuals with a detectable viral load (>200 copies/ml) in Philadelphia included in analyses. Being busy with personal things, like family work or difficulty getting to care, was the most commonly reported barrier to HIV care among individuals with a detectable viral load (30.1%), followed by mental health (21.7%), feeling well (17.2%), and problems with money or health insurance (11.8%). The PAF of financial barriers on detectable viral load was highest among non-Hispanic Whites (8.3%), with non-Hispanic Black and Latine having similar PAFs. The PAF of mental health on detectable viral load was highest among non-Hispanic Blacks (5.8%), with non-Hispanic White and Latine having similar PAFs. Non-Hispanic Black and Latine had similar PAFs of personal reasons on detectable viral load, but there was an inverse association between the barrier of personal reasons and detectable viral load among non-Hispanic Whites.

Conclusion: No single barrier to HIV care accounts for the plurality of individuals with a detectable viral load. System-level implementation strategies for increasing viral suppression will need to be tailored to specific populations with a health equity lens. Better access to mental health services and supportive services for retention in care may be the best strategies for increasing viral suppression among racial/ethnic minorities. Future research should assess the PAF of barriers in combination to optimize service delivery.

Table 1: Population attributable fractions for each barrier to HIV care by race/ethnicity

<table>
<thead>
<tr>
<th>Financial</th>
<th>Mental Health</th>
<th>Feeling Well</th>
<th>Personal Reasons</th>
</tr>
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<tbody>
<tr>
<td>Non-Hispanic White</td>
<td>6.8% (95% CI: 4.4, 9.7)</td>
<td>3.9% (95% CI: 2.1, 4.0)</td>
<td>3.9% (95% CI: 3.1, 4.8)</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>0.9% (95% CI: 2.1, 4.5)</td>
<td>5.6% (95% CI: 4.8, 6.5)</td>
<td>4.7% (95% CI: 3.8, 5.6)</td>
</tr>
<tr>
<td>Hispanic/Latine</td>
<td>3.4% (95% CI: 2.7, 4.2)</td>
<td>5.5% (95% CI: 4.6, 6.5)</td>
<td>3.7% (95% CI: 2.9, 4.7)</td>
</tr>
</tbody>
</table>

1184 Novel Post-Hospitalization Care Model Decreases Mortality in People with HIV in Zambia: Pilot Study

Cassidy W. Claassen, Chisivhu Nyamuwa, Marley Munjasi, Linah Mwango, Kirsten Strenereau, Caitlin Baumhart, Godfrey Muchanga, Brianna Lindsay, Mundai Mwirumwa, Nyuma Mbewe, Wilbroad Mutale, Michael Vinikoor
University of Maryland - College Park, College Park, MD, USA; University of Alabama at Birmingham, Birmingham, AL, USA; University of Zambia, Lusaka, Zambia; University of Lusaka, Lusaka, Zambia; University of Alabama at Birmingham, Birmingham, AL, USA

Background: Despite progress in HIV epidemic control in Zambia, HIV-related mortality remains high. Deaths are often preceded by hospitalization, and post-discharge mortality among people with HIV (PLWH) reaches 20-40% within six months due to individual, psychosocial, and systematic factors. We conducted a feasibility study of a community health worker (CHW)-led model to improve patient health outcomes and reduce mortality post-discharge.

Methods: A quasi-experimental feasibility and acceptability study was conducted at two tertiary hospitals in Lusaka, Zambia, using the PRISM implementation science framework. Adults hospitalized with HIV in Lusaka and then discharged were enrolled and followed up for 6 months post-discharge. The control group received standard of care (SOC) with telephonic follow-up. The intervention group received a novel care package, consisting of a discharge summary card, CHW home visits within one week of discharge, and screening and referral for depression and alcohol abuse at 1-3 months post-discharge. A physician-clinical liaison officer team based at the discharging hospital oversaw the CHW home visits, which included psychosocial counseling, vital signs check, medication counseling, and outpatient follow-up. Home visit data were collected by CHWs using electronic devices.

Results: Among 124 patients (median age, 41 years; 57.8% women; median CD4, 299 cells/mm³) in the SOC group, 23 (18.6%) died within 6 months of discharge. From 18 August to 20 September 2023, 21 patients enrolled in the pilot intervention group. To date, 13 (61.9%) received at least one home visit (7 of these were within 1 week of discharge) and 5 two visits, 15 (71.4%) received a discharge summary card, and 12 (57.1%) were screened for behavioral health problems. At one month, 12 were alive, 7 (7.7%) had died (from extrapolumonary tuberculosis), and 1 (7.7%) was readmitted based on the findings of the CHW at hospital visit. Acceptability among participants and caregivers has been high.

Conclusion: A novel discharge model of care, involving enhanced discharge instructions, CHW home visits, and screening and referral for behavioral health problems, appeared to be feasible and acceptable in urban Zambia. Post-hospital CHW visits have potential to reduce post-discharge mortality among PLWH in countries with generalized HIV epidemics such as Zambia. Focusing on the peri-discharge period can strengthen health systems as countries move into HIV epidemic control.

1185 The HIV Care Cascade in Medicaid, 2001-2015

Jacqueline E. Rudolph, Keri Calkins, Carinne E. Joshua, Bryan Lau
The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; Mathematica, Princeton, NJ, USA

Background: Medicaid serves as the largest single source of insurance for people with HIV (PWH) in the US, with approximately 40% of PWH covered by Medicaid. Not only does Medicaid represent a large, diverse population of PWH in US, it also represents some of the most vulnerable PWH, since eligibility is based on low income or disability. While the HIV care cascade is well characterized among all PWH in the US and among those linked to HIV care clinics, less is known about the state of the care cascade among PWH on Medicaid or how the cascade has changed over time.

Methods: We analyzed data from 273,799 Medicaid beneficiaries with HIV, enrolled in 14 US states, 2001-2015. All beneficiaries identified as having HIV through claims records were aware of their diagnosis and linked to care, hence, we focused on the later steps of the care cascade. We estimated prevalence of 4 levels of the HIV care cascade: retained in care and adherent to ART; retained but not on ART; not retained but on ART; not retained and not on ART. Beneficiaries were considered retained in care if they had an office visit, viral load measurement, or CD4 cell count every 6 months. Adherence to ART in each month of follow-up was defined as having a medication possession ratio of at least 80%. Prevalence of each state in each month 2001-2015 was estimated using a non-parametric multi-state approach, accounting for death as a competing event and for loss of Medicaid coverage using inverse probability censoring weights. Analyses were conducted overall and by US state.

Results: As shown in the Figure, 20% of beneficiaries with HIV were retained and ART adherent in 2001; this proportion reached a peak of 36% in 2013. The proportion not retained in care but ART adherent did not meaningfully change across follow-up (5-8%). In contrast, the proportion retained in care but not ART adherent decreased from 53% to 32%, and the proportion not retained in care and not ART adherent decreased from 20% to 13%. Death remained an important competing event in this era, with a cumulative incidence of 27% by 2015. Results differed by US state.

Conclusion: Despite being linked to care, less than half of beneficiaries with HIV were classified as ART adherent across all of follow-up, likely indicating that many Medicaid beneficiaries with HIV were not virally suppressed during this time period. These findings were seen even in the post-2012 “Treat All” era.
Future work will explore whether HIV care engagement improved between 2015-2021.

1186 Prevalence and Predictors of Advanced Disease Among People Living With HIV in Masaka Region, Uganda
Alex Daama1, Fred Nalugoda1, Anasi Kasango2, Betty Nantume1, Grace N. Kigozi1, Robert Ssekubug2, Abisalom Ssekituva1, Joseph Kagaayi1, David Servadda1, Joseph Kabanda1, Arthur G. Fitzmaurice1, Nelson Sewankambo1, Godfrey Kigozi1, Gertrude Nakigozi1, Nakali Health Sciences Program, Kalisizo, Uganda, 1Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: A large percentage (22%) of people living with HIV (PLHIV) present for care with advanced HIV disease (AHD), threatening the achievement of the 95–95–95 goals to end AIDS by 2030. For anyone over the age of five, AHD is defined as CD4 count ≤ 200 cells/mm3 or with a current WHO stage 3 or 4 events. This study aimed to examine the prevalence and factors related to AHD among newly diagnosed PLHIV in Masaka Region, Uganda.

Methods: A cross-sectional study was conducted from October 2021 through September 2022 among newly diagnosed PLHIV enrolled in care from 12 districts in Masaka Region. Data from electronic medical records (EMR) at health facilities were extracted for analysis. Variables included sex, age, marital status, location of facilities, and points of entry into care. Using a bivariable analysis, we determined the prevalence of AHD. A multivariable modified Poisson regression analysis was used to determine predictors with 95% confidence intervals (CIs).

Results: Of 3,452 newly diagnosed PLHIV on ART included in this study, 2,254 (65.3%) were females. The prevalence of AHD was 15% (518). The results from multivariable modified Poisson regression revealed that participants aged 18–35 years had lower risk of AHD compared to those aged 5–17 years (aPR=0.45; 95% CI: 0.27, 0.78). Married individuals were at lower risk of AHD compared to unmarried participants (aPR=0.67; 95% CI: 0.57, 0.79). Male participants (269/1,198, 22.5%) had higher risk of AHD compared to females (249/2,254, 11.1%; aPR=1.85; 95% CI: 1.57, 2.19). Participants receiving ART services from urban facilities had higher risk of AHD compared to participants receiving ART from rural sites (aPR=1.61; 95% CI: 1.35, 1.93). Participants who were enrolled into care through HIV testing service routes had lower risk of AHD while participants from other care points had lower risk of AHD (aPR=1.28; 95% CI: 1.06, 1.53) had higher risk of AHD compared to the general outpatient point.

Conclusion: The proportion of AHD in this cohort (15%) was lower than the national proportion of 22%. However, this work can be used to design interventions to address higher AHD prevalence among males, in urban facilities and points of entry into care through HIV testing service outreaches (aPR=0.72; 95% CI: 0.58, 0.90). Participants receiving ART services from urban facilities had higher risk of AHD compared to participants receiving ART from rural sites (aPR=1.61; 95% CI: 1.35, 1.93). Participants who were enrolled into care through HIV testing service routes had lower risk of AHD while participants from other care points had lower risk of AHD (aPR=1.28; 95% CI: 1.06, 1.53) had higher risk of AHD compared to the general outpatient point. The proportion of AHD in this cohort (15%) was lower than the national proportion of 22%

1187 Met and Unmet Health and Welfare Services Needs Amongst People With HIV in the UK
Anne Williamson1, Fiona Lampe2, Adamma Aghazua1, Anneget Pelchen-Matthews3, Alex Sparrowhawk1, Janey Sewell1, Clare Humphreys1, Alison Rodger1, Meaghan Kall1, Colette Smith1, for the PV2022 Study Group
1Guy’s and St Thomas’ NHS Foundation Trust, London, United Kingdom, 2University College London, London, United Kingdom, 3UK Health Security Agency, London, United Kingdom, 4Terrence Higgins Trust, London, United Kingdom

Background: Despite accessible and effective HIV treatment, the health and wellbeing of many people with HIV (PWH) is negatively impacted by social and economic disadvantage and unmet need for services. We assessed unmet health and welfare services needs in a large UK survey.

Methods: Positive Voices 2022 is the largest survey of PWH accessing care in the UK. Participants completed a questionnaire on demographics, socioeconomic status (money to meet basic needs), health and lifestyle factors, and unmet needs, and sexual behaviour. We analysed factors associated with increased need for services, defined as follows: (i) Mental health/drug services: psychological/stress support, alcohol/drug counselling, chemsex/ drug detox; (ii) Physical health: weight management, sex life support, smoking cessation, family planning, home services; (iii) Welfare: housing, meal services, employment/benefits/financial advice, legal/immigration support. We conducted multivariable logistic regressions to quantify odds of reporting any need, and the odds of any unmet need amongst those with need. Each model was adjusted for age, demographics, time since HIV diagnosis, and other covariates if significant in an adjusted analysis (Table 1).

Results: 4620 people participated; 2464 (53%) gay, bisexual and other men who have sex with men (GBMSM), 911 (20%) heterosexual women, 585 (13%) heterosexual men; 1117 (24%) were of Black ethnicity; median (IQR) age of 52 years (43–60). 1617 reported any mental health/driver service need, of whom 1066 (65.9%) had unmet need; 1879 any physical health need, of whom 1290 (68.7%) had unmet need; and 1454 any welfare need, of whom 1008 (69.3%) had unmet need. Table 1 shows all results. Need and unmet need were highest for younger people, except for unmet mental health need. Mental health need and unmet physical health need were higher for GBMSM, whilst physical health and welfare service needs were higher amongst Black African respondents. Lack of money to meet basic needs was associated with both mental and physical health needs, with mental health needs not met. Having depressive symptoms was associated with both physical health and welfare needs.

Conclusion: There is a significant burden of unmet health and welfare service need amongst PWH in the UK, despite universal healthcare access. HIV services should assess unmet need and identify routes to accessing available support, especially for younger people, those from minority groups, and those facing poverty or mental health challenges.

Junine Toy1, Raquel M. Espinosa1, Jason Trigg1, Tian Shen2, Paul Sereda3, Erin Ready1, Viviane Dias Lima1, Rolando Barrios1, Julia Montaner1
1British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada, 2St Paul’s Hospital, Vancouver, Canada

Background: Publicly funded, centrally distributed HIV PrEP with emtricitabine-tenofovir has been available in British Columbia (BC) since January 2018. We evaluated PrEP persistence and estimated medication utilization using longitudinal prescription data from BC’s PrEP program.

Methods: BC PrEP participants with ≥1 dispensed PrEP prescription (Rx) between 1-Jan-2018 to 30-Jun-2022 and ≥1 year follow-up opportunity were included. Demographics and PrEP Rx data were described, and PrEP persistence characterized by 1, 2 or ≥3 Rx. Multinomial logistic regression was used to obtain the univariate odds ratio (OR) to compare persistence in ≥3 vs. 1 Rx for variables of interest. For those with ≥3 Rx, PrEP utilization was estimated by calculating proportion of days covered (PDC), and stratified by prescribed daily vs. non-daily use.

Results: Overall, 9375 participants were included [median (Q1-Q3) age 32 (27-41) years; 96.9% cis-men, 1.3% trans-women, 0.9% cis-women, 0.5% trans-men; 54.3% reside in Vancouver]. 98.4%, 0.7%, and 0.2% qualified with men who have sex with men (MSM), heterosexual-, and injection drug use-based risk criteria, respectively. 80% (n=7520) of participants persisted with PrEP ≥3 Rx, while 9% and 11% received only 1 and 2 Rxs, respectively. A significant difference in the odds of PrEP persisting ≥3 vs. 1 Rx was observed in several subgroups: age category 18-28 years (Ref. ≥48 years) (OR 0.7 [95% CI, 0.5-0.8], p=0.0002); gender cis-women (Ref. cis-male) (OR 0.2 [0.1-0.3], p<0.0001); trans-women (OR 0.3 [0.2-0.4], p<0.0001); trans-men (OR 0.4 [0.2-0.8], p<0.0001).
Community and Facility-Based PreP Uptake and Adherence Among People Who Use Drugs in Uganda

Joseph Kibuuka1, Patricia M. Smith1, Timothy Mwumange2, Peter Mudiwope1, Liz Komuhangi1, Ritah Kansime1, Tara Wood1, Florence Nambi1, Mai Nakumbende1, Lylianne Nakabugo1, Herbert Kadama1, Peter Krambaddde1, Sara N. Glick1, Andrew Mujugira1, Renee Heffron1

1Infectious Disease Institute, Kampala, Uganda, 2University of Alabama at Birmingham, Birmingham, AL, USA, 3Infectious Diseases Institute, Kampala, Uganda, 4Ministry of Health Uganda, Kampala, Uganda, 5Makerere University College of Health Sciences, Kampala, Uganda, 6National AIDS Control Program, Dar es Salaam, United Republic of Tanzania, 7University of Washington, Seattle, WA, USA

Background: People who use drugs (PWUD) in Uganda experience a high HIV burden and limited access to HIV prevention services. Integrating HIV pre-exposure prophylaxis (PrEP) into harm reduction services could substantially enhance service uptake and reduce risk of HIV acquisition.

Methods: From January 2022-August 2023, we enrolled PWUD into TDF-based oral PrEP programs integrated into harm reduction services at a community-based needle and syringe program (NSP) and a facility-based medication-assisted treatment (MAT) program located in Kampala, Uganda (NCT05040308). The 6-month PrEP refill data were abstracted from medical records to estimate PrEP persistence. We enrolled a subset of participants into a research cohort to measure tenofovir concentration levels in plasma to estimate PrEP adherence. We compared the frequency of PrEP persistence and adherence across program type using log binomial regression models.

Results: Through August 2023, 100% of HIV-negative NSP clients (n=265) and 98.9% of MAT clients (n=91) with 6 months of follow up initiated PrEP. The median age was 31 (IQR 25-39), 90.7% were male, 63.7% reported using street opioids with injection and smoking being the most common route of use. Among 64% (n=227/355) of times when 1 bottle (30 pills) of PrEP was dispensed, a refill was sought within 30 days. When 60 pills were dispensed, 65% of participants returned within 60 days and when 90 pills were dispensed, 45% returned within 90 days. Six-month PrEP persistence in MAT and NSP was 12% and 62% (relative risk=0.16, 95% CI: 0.09-0.27, p<0.0001). In the subset using PrEP, 6-month plasma TFV levels were high (>31ng/ml) in 52% and moderate (0.1-31ng/ml) in 37% of NSP participants and high in 34% and moderate in 32% of MAT participants (global p-value p=0.07).

Conclusion: In integrated PrEP and harm reduction programs, PrEP uptake was very high. We observed greater PrEP persistence in the community-based NSP program and moderate adherence overall. Since these programs serve people with different harm reduction preferences, further research is needed to explore how to effectively integrate PrEP into community- and facility-based programs.

PreP Persistence and Ongoing HIV Risk Among Patients Initiating PreP at an Urban Malawian STI Clinic

Sarah E. Rutstein1, Jane Chen1, Esther Mathiya1, Griffin J. Bell2, Beatrice Hlabalala1, Tapiwa Munthali1, Naomi Nyirenda1, Naumi Bonongwe1, Claire Pedersen1, Edward Jere1, Mina Hosseinipour1, Zakalah Mpande1, Irving F. Hoffman1, Mitch Matoga5, for the ePrEP Study Team

1University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 2University of North Carolina Project–Malawi, Lilongwe, Malawi, 3Malawi Lionheart Trust, Lilongwe, Malawi

Background: People presenting with sexually transmitted infections (STIs) are a priority group for HIV prevention, including biomedical pre-exposure prophylaxis (PrEP). Longitudinal risk and persistent use of oral PrEP among STI patients in sub-Saharan Africa (SSA) has not been well characterized.

Methods: We enrolled men and women ≥15 years presenting to an urban STI clinic who were eligible for and initiated on PrEP according to Malawi PrEP guidelines. All PrEP care was provided by Ministry of Health staff. Participants were tested for Chlamydia trachomatis (Ct), and Neisseria gonorrhoeae (Ng) at enrollment, month 3, and month 6, and completed behavioral surveys. PreP persistence was derived from PrEP refill review, defined as no ≥7 days without pills based on visit date, pills distributed, and self-reported missed pills.

Results: We enrolled 174 participants initiating PrEP between March–December 2022. Nearly two-thirds were male (109/174; 63%), and 70/174 (40%) were <25 years. In the month preceding PrEP initiation, 23/174 (13%) reported having a partner living with HIV and 69/174 (40%) had exchanged sex for goods, money, or favors. Four (4%) men endorsed ever having anal sex. Of 173 with linked PrEP records, 66 (38%) were persistent on PrEP at 6-months, 58 (34%) were not persistent but retained in the study, and 49 (28%) were not persistent and lost to follow-up. Approximately half (94/173; 54%) were persistent at 3 months. 6-month persistence was higher for women (29/65; 45%) vs men (37/108; 34%) and for those aged ≥25 (43/103; 42%) vs <25 (23/70; 33%). Persistent HIV risk was common among those retained in the study, regardless of PrEP persistence. Among those with ≥1 follow-up study visit (153/174; 88%), 25 (16%) reported having a partner living with HIV, and 58 (33%) reported exchanging sex for goods, money, or favors in the preceding month. Among the 136/174 (78%) with ≥1 follow-up Ct/Ng test, 32 (24%) had ≥1 incident STI (Ct: 15; Ng: 7, Ct and Ng: 10).

Conclusion: Despite ongoing HIV risk, including frequent incident STIs in a short follow-up period, PrEP persistence among a cohort of persons initiating PrEP from an STI clinic in Malawi was poor. Persistence was particularly low among men, who accounted for nearly two-thirds of the study population.
1192  PrEP Knowledge and Use: Results from the Zambia Population-Based HIV Impact Assessment 2021

Nzali G. Kancheya, Brian Muyunya, Omega Chitsulo, Megumi Itoh, Bupe Musonda, Mumbi Chola, Megan Bronson
1Centers for Disease Control and Prevention, Lusaka, Zambia, 2Government of Zambia Ministry of Health, Lusaka, Zambia, 3University of Maryland, Baltimore, MD, USA, 4Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** Pre-exposure prophylaxis (PrEP) taken daily has been shown to be effective prevention against HIV acquisition and has been recommended by WHO for people at substantial risk for HIV since 2015. Zambia has been implementing the PrEP program since 2017 and rolled it out nationwide in 2018.

**Methods:** We analyzed data from the 2021 Zambia Population Based HIV Impact Assessment (ZAMPHIA). We used descriptive analysis (with chi-square test) to analyze demographic and behavioral characteristics of PrEP knowledge and PrEP use, and a weighted multivariable logistic regression model to identify key predictors of PrEP use among all participants. All results were weighted to account for complex survey design.

**Results:** Among 22,183 participants aged 15+ year who answered the PrEP questions, 21.2% (95% CI: 20.3-22.2) reported having ever heard of PrEP: 18.9% (95% CI: 17.6-20.2) among men and 23.5% (95% CI: 22.3-24.8) among women. Within age groups 17.5% (95% CI 16.1-18.9) of young adults aged 15 to 24 years and 17.2% (95% CI 15.8-18.7) of those aged 45+ years had ever heard of PrEP. Other associations characterized with having ever heard of PrEP were being married (22.1% [95% CI: 20.9-23.3]), living in urban areas (30.3% [95% CI:28.5-32.1]) and having more than a secondary education (56.7% [95% CI:52.8-60.4]). Of those who have ever heard of PrEP, 3.9% (95% CI: 3.2-4.7) reported ever using PrEP. PrEP use was highest among those who reported having a sexual partner who is HIV positive 16.5% (95% CI: 12.3-21.7). Those who reported a HIV positive sexual partner were more likely to report ever having used PrEP compared to those who did not know the HIV status of their partner (aOR 5.84 [95% CI: 3.27-10.41]; P<0.001).

**Conclusion:** Although PrEP has been available country wide since 2018, just over one-fifth of adult ages 15+ years were aware of PrEP. This is of concern particularly among young adults aged 15-24 who have the highest incidence of HIV as seen in ZAMPHIA. These results can guide the Ministry of Health to target the dissemination of PrEP information to populations with low knowledge of PrEP, including men, those aged 45+ years, living rural areas, and with lower education levels. These results demonstrate that having a sexual partner who is HIV positive is a strong predictor for ever having used PrEP. More efforts, however, are needed to increase uptake in other populations that are at substantial risk for HIV infection, such as persons with multiple sexual partners and low condom use.

**Table 1.** Sexual health outcomes among once-daily and on-demand PrEP users in China

<table>
<thead>
<tr>
<th></th>
<th>On-demand (n=385)</th>
<th>Once-daily (n=1,804)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condom use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always to nearly never</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90.1%</td>
<td>35.1%</td>
<td>8.0%</td>
<td></td>
</tr>
<tr>
<td>Infrequent use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64.1%</td>
<td>15.1%</td>
<td>4.5%</td>
<td></td>
</tr>
<tr>
<td>Often use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.8%</td>
<td>15.1%</td>
<td>4.5%</td>
<td></td>
</tr>
<tr>
<td>Sex under influence of substances use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol, drugs, inhalants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69.1%</td>
<td>45.9%</td>
<td>1.6%</td>
<td></td>
</tr>
<tr>
<td>Sex under influence of substances use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol, drugs, inhalants</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>69.1%</td>
<td>45.9%</td>
<td>1.6%</td>
<td></td>
</tr>
<tr>
<td>New diagnoses</td>
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<td></td>
</tr>
<tr>
<td>STI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.4%</td>
<td>5.0%</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td>STI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.4%</td>
<td>5.0%</td>
<td>1.0%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** Demographic and Behavioral Characteristics Associated with PrEP use among Sexual Partners in ZAMPHIA 2021 (Weighted)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Number</th>
<th>P-value</th>
<th>Number</th>
<th>P-value</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever heard of PrEP</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22,183</td>
<td>14,917</td>
<td>&lt;0.0001</td>
<td>14,917</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>7,271</td>
<td>2,369</td>
<td>0.2509</td>
<td>2,369</td>
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<tr>
<td>Sex partner HIV status</td>
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<tr>
<td>HIV positive</td>
<td>2,441</td>
<td>1,436</td>
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<tr>
<td>HIV negative</td>
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<td>13,481</td>
<td>&lt;0.0001</td>
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<tr>
<td>Gender</td>
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</tr>
<tr>
<td>Male</td>
<td>11,419</td>
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<tr>
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<td>Asian</td>
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</tr>
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<td>&lt;0.0001</td>
<td>2,509</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1193  Sexual Health Outcomes Among Daily and On-Demand Oral PrEP Users in China

Chunyan Li, Zhihoug Yin, Weiming Tang, Songjie Wu, Quanmin Li, Yifan Dai, Chengxin Fan, Ke Liang, Linghua Li, Wu Sun, Joseph D. Tucker, Haojie Huang, Jonathan Loi, Aniruddha Hazra, Renslow Sherer
1University of Tokyo, Tokyo, Japan, 2University of North Carolina, Guangzhou, China, 3University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, 4Zhongshan Hospital of Wuhan University, Guangzhou, China, 5Guangzhou Eighth People’s Hospital, Guangzhou, China, 6Wuhan Tongying LGBT Center, Guangzhou, China, 7University of Chicago, Chicago, IL, USA

**Background:** Longitudinal data on sex behaviors and STIs among Chinese PrEP users is limited. Here we reported self-reported newly diagnosed STIs and sex behaviors among MSM who are on daily and on-demand PrEP regimens in China.

**Methods:** MSM in Wuhan and Guangzhou were recruited by online ads, clinic flyers, and community referrals to a PrEP demonstration trial. Behavioral survey data were collected at baseline and quarterly follow-ups over 12 months (M3, M6, M9, M12). MSM were prescribed TDF/TFC as oral PrEP and could opt for daily or on-demand regimens. Descriptive analysis and multivariable logistic regressions were conducted in Stata 15.0.

**Results:** From September 2021 to September 2023, 1,209 MSM were enrolled and initiated PrEP (mean age=28.0, IQR 20.4-31.4), with 164 (16.0%) dropping out before the trial ended. About half of the participants opted for daily PrEP, while the rest chose on-demand (2+1+1). MSM reporting not having penetrative sex during the three months increased from 3.9% at baseline to 11.1% at M12 (p<0.001). Regarding condom use behaviors, we observed a notable increase of MSM reporting “never to seldom using condoms in sex” at M9 & M12 (17.3%, 18.6%) compared to baseline (10.1%) (p<0.001). The proportion of people having sex under the influence of substance use (predominantly alcohol & nitrate inhalants) remained consistently high (52.8% at baseline vs. 46.0% at M12). The proportion of people who self-reported any STI diagnosed in the past 3 months remained 4.9 to 8.0% during the study period, with the sulphur prevalence stabilizing from 3.6-5.0%. Multivariable analyses indicated that PrEP dosing strategies were not associated with newly diagnosed STIs, condom use habits change, or sex under the influence of substances over time. However, MSM of higher income levels (OR=3.9, 95% CI: 1.7-9.1) and those who seldom to not use condoms (OR=3.5, 95% CI: 1.8-6.7) were more likely to report having sex while using substances.

**Conclusion:** Our study results showed comparable impact on risks of STIs or sex behavior changes between once-daily and on-demand oral PrEP dosing strategies. Nonetheless, the observed increased number of MSM not having sex, less condom use habits, and persistent prevalence of engaging in sex activities under the influence of substance use underscore the evolving sexual behavior patterns within Chinese MSM PrEP users. Engaging PrEP users in routine testing and safe sex counseling is critical to the holistic health promotion of MSM communities.

1194 HIV Recency Surveillance Improves PrEP Uptake: Evaluation With Synthetic Controls

Sara Wallace, Matthew R. Lamb, Claire Steiner, Eugenie Peroit, Samkelo Simelane, Dumile Sibandze, Vusie Loketfwako, Aisha Pasha, Harriet Nwagaba-Birbonwoh, Yen T. Duong, Melissa Arons, Munyaradzi Pasipamire, Suzue Saito, Lenhle Dube, Samkelo Simelane, Claire Steiner
1Columbia University, New York, NY, USA, 2ICAP at Columbia University, New York, NY, USA.

**Background:** Eswatini’s HIV-1 Recent Infection Surveillance (EHIS) program, implemented since 2019, identifies trends in recent and long-term HIV infections. EHIS flags hotspots (HIV testing centers with ≥4 monthly recent infections) for public health responses focused on fidelity to index testing, partner notification, and linkage to PrEP for contacts testing negative. We used
synthetic control methods (SCM) to evaluate the impact of this public health response on PrEP enrollment. **Methods:** We used October 2019–December 2022 data to conduct a difference-in-difference analysis with SCM to compare trends in PrEP enrollment between intervention (n=9) and non-intervention facilities (n=93) pre- and post-intervention. SCM is a data-driven technique that manufactures a weighted synthetic control (SC) from a pool of possible controls to best approximate pre-treatment trends observed in the intervention unit(s). The intervention point was August 1, 2020. Pre-intervention trends were estimated using facility-level characteristics associated with historic PrEP enrollment trends. To ensure the SC did not create differences unrelated to the intervention, we completed an "in-time" placebo test, using March 1, 2021 as our fake intervention time. **Results:** Similar pre-intervention trends were observed in the intervention clinics and the SC (Figure 1a). The average treatment effect for the post-intervention period was 10.2 additional PrEP enrollees a month, from an average of 6.7 in the SC to 16.9 in the intervention (p=0.004). Six months post intervention, PrEP enrollment increased in both groups but increased more in intervention facilities; this was sustained during the rest of the study period. Between April–December 2022, intervention clinics averaged roughly twice the monthly PrEP enrollment of the SC. Decreases in June 2021 in both arms occurred during a period of political unrest. The mean squared prediction error for the model was 3.41, demonstrating adequate predictive accuracy of the model for program data. The placebo test (Figure 1b) showed no difference in outcome trends unrelated to the intervention. **Conclusion:** EHRs-informed interventions resulted in increased and sustained PrEP enrollment in intervention facilities in Eswatini compared to the SC. Recency data strengthened linkages to PrEP services among HIV negative contacts and increased PrEP uptake.

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**1195 Expanding Event-Driven PrEP to People Assigned Male at Birth Across 3 African Countries**

Lirica Nishimoto1, Augustine Amedzi1, Makhosazana Matsebula2, Bhekizitha Sihole1, Rachel Lyimo1, Hawkins Reeves1, Gertrude Nunu2, Ncamsile Dlamini2, Gift Kamanga3, Michael Ode4, Nana Fousa Clement5, Tiffany Lille6, Christina Fischer-Walker6, Chris Akolo1

1FHI 360, Washington, DC, USA, 2FHI 360, Accra, Ghana, 3FHI 360, Mbabane, Eswatini, 4FHI 360, Monrovia, Liberia

**Background:** In 2022, World Health Organization recommended the expansion of event-driven pre-exposure prophylaxis (ED-PrEP) to prevent HIV not only among men who have sex with men (MSM) but also all people assigned male at birth (AMAB) who are not using estradiol-based exogenous hormones. The USAID- and PEPFAR-funded Meeting Targets and Maintaining Epidemic Control (EpiC) project offered ED-PrEP as another option to oral daily PrEP to people AMAB not using estradiol-based exogenous hormones across three sub-Saharan African countries.

**Methods:** Using various differentiated service delivery (DSD) models, EpiC offered HIV-negative people AMAB who are MSM, who purchase sex, who are transgender, and other people AMAB options for daily PrEP and ED-PrEP in Ghana, Liberia, and Eswatini. ED-PrEP was recommended for people who had infrequent and predictable sexual encounters or anyone who wanted to enroll. Routinely collected programmatic data was analyzed to understand preference and uptake by population and age groups across the three countries.

**Results:** Between October 2021 and May 2023, EpiC newly initiated 8,495 individuals AMAB on oral PrEP in the three countries, among whom 7,101 (84.40%) initiated on ED-PrEP. A larger proportion of people AMAB who were not MSM chose ED-PrEP over traditional daily PrEP (95.36%) compared to MSM (76.85% chose ED-PrEP). The proportion of those who initiated ED-PrEP over daily PrEP was highest among individuals 40–44 years of age (95.20%); 536/569 and lowest among those 50 and over (49.72%; 90/195) (Figure 1) Oral PrEP uptake among people AMAB who were not MSM (84.58%) was higher than among MSM (75.92%). PrEP uptake among those to whom it was offered increased from 39.99% (October 2020–September 2021) to 72.57% (October 2021–May 2023) after ED-PrEP was offered in Ghana and Eswatini. The increase was most pronounced among those aged 50 year and older, from 18.95% to 75.58%.

**Conclusion:** The results demonstrate the feasibility of offering ED-PrEP to all people AMAB and the preference of some of these individuals for ED-PrEP compared to daily oral PrEP. Scale-up of ED-PrEP may improve oral PrEP uptake among all people AMAB warranting ED-PrEP expansion efforts beyond MSM. More efforts are needed to improve access to ED-PrEP through various DSD models as we scale oral PrEP provision. More research is needed to understand preferences for all individuals AMAB—not just MSM—and to improve uptake strategies.
**1197 High Individual- and Community-Level Variability in Male Circumcision Coverage in Rakia, Uganda**
Hadija Nakawooya1, Mary Kate Grabowski, Victor Siempjja, Robert Ssekubugya, Anthony Ndyabanjo, Joseph Kagayji, Arthur G. Fitzgerald, Ping T. Ye7, Larry W. Chang, Aaron A. R. Tobian, Ronald H. Gray, Maria J. Wawer, David Serwadda, Steven J. Reynolds5, Godfrey Kigozi2
1Rakai Health Sciences Program, Rakia, Uganda, 2US Centers for Disease Control and Prevention Kampala, Kampala, Uganda, 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 4The Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Background:** Voluntary male circumcision (VMMC) is a proven intervention for reducing HIV incidence in men. Identifying specific populations and geographic areas with low VMMC coverage for targeted service outreach may help prioritize limited resources and achieve HIV epidemic control.

**Methods:** We used data from the Rakai Community Cohort Study, a population-based HIV surveillance cohort in Uganda, collected June 2018–November 2020, to assess individual and community-level factors associated with MC coverage among non-Muslim HIV-negative men across 39 communities. Muslims participants were excluded because circumcision is routine religious practice. Individual factors assessed included age, marital status, religion, education level, and past year number of sexual partners. Community-level factors included community type (agrarian, trading, and Lake Victoria fishing), percentage of population <30 years, percentage with primary education or less, percentage Muslim, average distance of households to the nearest health facility (level III/IV), average distance of households to a main road, and HIV seroprevalence. Hierarchical multivariable Poisson regression with community random effects was used to estimate individual- and community-level associations with MC coverage.

**Results:** Of the 8,757 men in the cohort, 86.4% (7,570) identified as non-Muslim, and 85.2% of these (n=6,448) were HIV-seronegative and enrolled in the study; Muslims were excluded from this analysis due to the expected full MC coverage as a religious practice. Of those enrolled, 61.7% were circumcised. Being circumcised was strongly associated with age, with 77% of boys and young men 15–24 years circumcised, compared to 51% of men 40–49 years old (prevalence ratio (PR)=0.64, 95% CI: 0.59–0.70). Median community coverage of MC was 66% ranging from 32% to 88% across communities. MC coverage was lower in communities with a greater percentage of people living with HIV (adj. PR=0.87,95% CI: 0.77–0.97), MC coverage decreased with greater distances to the main road (adj. PR=0.81, 95% CI: 0.69–0.95) and non-significantly with health facilities. Communities with larger Muslim populations had significantly higher MC coverage among non-Muslim HIV-negative men (adj. PR=1.13, 95% CI: 1.04,1.23).

**Conclusion:** We observed individual and community-level variation in VMMC coverage among HIV-negative non-Muslim men. Prioritizing VMMC services in communities with higher HIV burden and limited health facility access may improve VMMC coverage.

The figure, table, or graphic for this abstract has been removed.

**1198 Increased Viral Suppression With Adherence Counseling Incorporating a Point-of-Care Urine TFV Test**
Leonard T. Bikinesi1, Matthew A. Spinelli,5 Nombizodwa M. Nyoni,7 Jesaya Hifindwako,2 Assegid Mengistu1, Jacques Kamangui,3 Gram Mutandu,4 Daniella Mouton,4 Fekir Negusie1, Rachel S. Beard,1 Ingrid Katz2
1Ministry of Health and Social Services, Windhoek, Namibia, 2University of California San Francisco, San Francisco, CA, USA, 3Namibia Institute of Pathology, Windhoek, Namibia, 4Centers for Disease Control and Prevention, Atlanta, GA, USA, 5US Centers for Disease Control and Prevention Windhoek, Windhoek, Namibia

**Background:** Innovative approaches are needed to achieve the third UNAIDS 95-95-95 target, to increase and sustain virologic suppression (VS) in patients on ART, specifically co-formulated tenofovir (TFV)-lamivudine–dolutegravir (DTG) or TLD. Virologic failure in patients on TLD is likely due to non-adherence because of DTG's high resistance barrier. Identifying non-adherence to TLD with a point-of-care metric and tailored counseling on the test may help patients achieve viral suppression (VS). We integrated a low-cost, POC urine test to detect TFV into standard WHO-recommended enhanced adherence counseling (EAC) to improve VS in adults with non-VS on TLD in Namibia.

**Methods:** Patients on TLD with viral load (VL) >1000 copies/mL after completing ≥1 round of EAC were enrolled from 42 clinics across Namibia. At each monthly ART pick-up, participants completed the POC urine test and received EAC informed by test results. After 3 months (round 1), participants received a viral load (VL) test. If VS was not achieved, up to 3 additional rounds of POC urine testing with EAC was provided, with an HIV drug resistance test sent at month(M) 9. Acceptability of the urine assay was assessed via surveys administered to participants and providers.

**Results:** Of 211 participants enrolled (median age 33 years, interquartile range 22–46, 61% female), 195 reached M3 and received a follow-up VL, with 169 (87%) achieving VS within M3 and 182 (93%) by M9. Moreover, in those who achieved VS, positive TV in urine increased from 81% at baseline to 96% at M9 compared to a change from 31% to 47% among unsuppressed individuals. Drug resistance testing was performed in 5 remaining participants with high VL at M9. All 5 had variable urine TFV results after visits and one had DTG resistance (N155H and R263K mutations). Overall, 84% of participants and 89% of interviewed providers agreed/strongly agreed that the urine test improved EAC.

**Conclusion:** Nearly 90% of patients on TLD with VL >1000 copies/mL achieved VS within 3 months (93% at M9) following EAC that incorporated a urine-based POC TFV test, compared to 33% of individuals receiving 1-3 rounds of standard WHO-recommended EAC. Encouraging results of this pre-post intervention support rigorous testing in a future randomized clinical trial. Given the cost of VL and resistance testing in lower and middle-income countries, this POC urine test has great potential to help achieve the third 95-95-95 target in a low-cost, scalable manner.

**1199 Persistent Challenges With Viral Suppression a Year After Return to Care: Evidence From South Africa**
Claire M. Keene4, Jonathan Euvrard1, Tali Cassidy3, Mike English,4 Jacob McKnight4, Catherine Orrell5, Ingrid Katz2
1University of Oxford, Oxford, United Kingdom, 2University of Cape Town, Cape Town, South Africa, 3Harvard Medical School, Boston, MA, USA

**Background:** Even though 94% of people living with HIV in South Africa knew their status in 2021, only 74% were actually on antiretroviral therapy (ART) and 67% were virologically suppressed: undermining the potential of ART programmes to improve individual and public health outcomes. Understanding which populations drive this poor treatment success could focus efforts to improve outcomes.

**Methods:** This retrospective study describes the incidence and impact of ART treatment interruptions using routine health data from the Provincial Health Data Centre, South Africa. The cohort includes individuals ≥15 years old who initiated ART under universal test-and-treat (≥01-Sep-2016), sought care in Khayelitsha or Gugulethu (low-resource, high HIV burden settings) and had ≥1 year follow-up. Treatment interruptions were defined as >90 days late for an expected visit (based on duration of treatment dispensed) or being lost to follow-up (no visit within 180 days of database closure on 30-Sep-2022). One-year retention was defined as a visit 9–15 months after ART initiation or restart, and suppression as a viral load (VL) ≤50 copies/mL.

**Results:** The cohort included 68888 individuals, with 69% (47631/68888) female, 25% (17078/68888) under 25 years and a median follow-up time of 4 years (Interquartile Range [IQR] 3–5). The cumulative incidence of interruptions was 71% (95% Confidence Interval [CI] 71–72) by 5 years after initiation, with a median of one interruption (IQR 1–2) and a median of 4 months (IQR 1–9) to interruption after initiation or restart (Figure 1). Most returned to care (cumulative incidence of return: 73% [95% CI 72–73] by 5 years), after a median of 7 months (IQR 4–15). Of the 40384 individual interruptions, there was sufficient follow-up to time to evaluate outcomes a year after re-engagement for 29967 (74%); 67% (20030/29967) were retained and 29% (8578/29967) had a VL that was suppressed year after return. In the 34% (23578/68888) who never had a treatment interruption, 73% (17201/23578) had a suppressed VL a year after ART initiation.

**Conclusion:** Treatment interruptions are common. Even after reengagement, those with interruptions have poor treatment outcomes. Health services need to improve care after return to better support retention and adherence, particularly in the first six months after ART restart. People returning to care should be a priority population for the development of differentiated service options, if we are to improve retention and VL suppression for the whole population on ART.
1200  Durability of Viral Suppression Among People on HIV Treatment in S Africa: A National Cohort Study

Jacob Bor1, Evelyn Laren1, Dickman Gareta2, Khumbo Shumba2, Mazvita Muchengeti3, Wendy Stevens4, Dorina Onyso5, Noleka Miliana1

1Boston University, Boston, MA, USA; 2Health Economics and Epidemiology Research Office, Johannesburg, South Africa, 3National Health Laboratory Service, Johannesburg, South Africa

Background: People on HIV treatment with a viral load (VL) < 200 copies/ml cannot transmit HIV sexually: “Undetectable = Untransmittable” (U=U). However, the utility of U=U as a prevention strategy has been questioned due to concerns about viral blips and viral rebound. We assessed durability of viral suppression in a national longitudinal cohort in South Africa.

Methods: Data were obtained from the National Health Laboratory Service (NHLS) National HIV Cohort. The NHLS Cohort, created through deduplication of South Africa’s national laboratory database, includes all viral loads in the public sector HIV program. People living with HIV (PLHIV) aged 15-59 years were included in the analysis if they had at least one VL <200 copies/ml between March 2015 – September 2016. PLHIV entered the study on the date of their first VL<200 and were followed for 18 months. PLHIV were defined as “monitored at 12 months” if they had any VL test 6-18 months after baseline. Durability of viral suppression was defined based on the value of the 12-month VL, reported in three categories: <200 copies/ml (zero transmission risk), 200-999 copies/ml (minimal transmission risk), and ≥1000 copies/ml (elevated transmission risk). Analyses were stratified by age, gender, province, and viral load history reflecting prior experience in HIV care.

Results: Of 2,383,871 PLHIV with VL<200 copies/ml at baseline, 73% were virally monitored at 12 months. Of those, 87% had a 12-month VL <200 copies/ml and 95% had a 12-month VL <1000 copies/ml. PLHIV whose VL at entry was their first ever VL had the lowest probability of being monitored at 12 months, at 60% (Fig 1). Those with a history (>2 years) of viral suppression had the highest rates of 12-month viral monitoring (>84%) and suppression (>97%) (Fig 1). Individuals with previous VLs between 200-999 or >1000 copies/ml faced the greatest challenges remaining suppressed (Fig 1). VL monitoring rates were lowest among PLHIV under 35 years, but among those monitored, durability of viral suppression was relatively consistent across age and gender. Monitoring rates were similar across provinces, with higher variability in durability of viral suppression.

Conclusion: 95% of PLHIV with VL<200 who returned for their 12-month VL had zero risk or minimal risk of transmission one year later. Our results highlight the importance of regular monitoring and the durability of viral suppression for PLHIV who remain in clinical care.

1201  Motivational Interviewing Training Effect on HIV Viral Suppression: A Cluster Randomised Pilot Trial

Dorina Onyso1, Tembeka Sineke1, Idah Mokhela1, Robert A. Ruiter1, Marnie Vujovic1, Khumbo Shumba1, Jacqui Miot1

1Health Economics and Epidemiology Research Office, Johannesburg, South Africa, 2Maastricht University, Maastricht, Netherlands, 3Anova Health Institute, Johannesburg, South Africa

Background: We developed a Motivational Interviewing (MI) skills training program for lay counsellors (The Thusa Thuso program) to improve the quality of patient-centred HIV retention counselling in South Africa (SA). We report the effect of the Thusa Thuso intervention on HIV patient outcomes at 12 months after HIV diagnosis.

Methods: We randomized eight primary healthcare clinics (PHC) in Johannesburg (SA) to either the intervention condition (n=4 clinics) where all lay counsellors were supported for 12 months before the patient enrolment or the standard of care support (n=4 clinics). Recorded Motivational Interviewing Treatment Integrity (MITI) coding of training was used to determine counsellor MI technical skills level (Cultivating Change Talk (CCT), Softening Sustain Talk (SST), Empathy and Partnership towards clients. A total of 554 adult (≥ 18 years) patients were recruited immediately after HIV diagnosis from March 2020 to August 2021 (n=293 intervention, n=261 control). We conducted cluster-adjusted Poisson regression to assess the intervention effect on retention in care (being within 90 days after a scheduled appointment) and viral suppression (<50 copies/ml) at 12 months after HIV diagnosis, reporting risk ratios (RR) with 95% confidence intervals (CIs).

Results: We observed modest retention rate improvement in patients supported by intervention clinic counsellors compared to control patients (RR 1.3, 95% CI: 0.9-2.0) and a marked increase in retention rates proportional to increasing MI skill levels (Fig 1) (CCT, RR 1.6, 95%CI: 1.1-2.5; SST, RR 1.5, 95%CI: 0.8-2.9; Empathy, RR 1.4, 95%CI: 1.0-1.8; Partnership RR 1.5, 95%CI: 1.2-1.9). Viral suppression at 12 months also increased in the intervention group (RR 1.4, 95%CI: 1.1-1.8). Similarly, to the retention outcome, increasing counsellor MI skills resulted in higher patient VL suppression at 12 months (CCT, RR 1.2, 95%CI: 1.2-1.3; SST, RR 1.3, 95%CI: 0.8-2.2; Empathy, RR 1.1, 95%CI: 0.9-1.4; Partnership, RR 1.2, 95%CI: 1.1-1.3).

Conclusion: Appropriately tailored MI training for lay counsellors, resulting in documented improvement in counsellor skills, can improve retention and VL suppression outcomes. While these preliminary data indicate potentially significant effects, statistical significance is limited by the pilot’s small cluster sample size and statistical power.

1202  Undisclosed ART Use Found by Urine LFA Is Common in South Africa, Not Associated With Lower TB Risk

Nsika N. Sithole1, Indira Govender1, Matthew A. Spinelli2, Theresa Smit3, Meaghan Krows4, Connie Celem1, Alison Grant1, Monica Gandhi5, Adrienne E. Shapiro6

1Africa Health Research Institute, Mkhuluza, South Africa, 2London School of Hygiene & Tropical Medicine, London, United Kingdom, 3University of California San Francisco, San Francisco, CA, USA, 4University of Washington, Seattle, WA, USA

Background: People with HIV (PWH) not on ART are at high risk for TB and low CD4 counts. TB screening and CD4 count measurement are recommended at ART initiation or re-initiation. In South Africa, estimated undiagnosed TB prevalence is 5-10% in PWH not on ART. An interim analysis of an ongoing cohort study of intensive TB screening in ART initiators found <1% TB prevalence. We used a urine tenofovir (TFV) assay to objectively assess the prevalence of undisclosed ART use as a potential explanation of the low TB prevalence in the cohort.

Methods: Adults with confirmed HIV reporting no ART use within 90 days had CD4 count testing and were systematically tested for TB at the time of ART initiation at 2 public clinics in KwaZulu-Natal, South Africa using sputum TB culture, sputum Xpert Ultra and urine TB-LAM testing.
Remaining urine was frozen and stored. Participants were followed for >6 months to identify incident TB. We tested thawed urine samples for TFV, indicating undisclosed ART use, using a novel lateral-flow urine assay (96% sensitive, 100% specific vs. LC-MS/MS). TB was defined as a positive Xpert Ultra or culture, or initiation of TB treatment within 3 months. We assessed predictors of ART nondisclosure and the relationship between ART nondisclosure and TB.

**Results:** Between 12/2021-8/2023, 315 PWH (126, 40% male) reporting no ART use presented for ART initiation and provided urine; of these 63 (20%) had detectable TFV indicating undisclosed ART use. Median CD4 count among persons with vs. without undisclosed ART was 503 cells/mm³ (IQR 280-637) vs. 336 (IQR 178-510; p=0.001). Undisclosed ART was more common in PWH attending a rural vs. a peri-urban clinic (35% vs 12.5%, p<0.001). In a multivariable model, undisclosed ART was associated with older age, rural clinic site, and increasing CD4 count, but not with gender, education, or employment. TB screening identified 15% (5% PWH with undiagnosed TB (11/15 male, 15/15 reporting TB symptoms, 8.7% prevalence in men). There was weak evidence for undisclosed ART being more common in persons with undiagnosed TB, after adjusting for gender (OR 2.70, 95% CI 0.91-8.03).

**Conclusion:** In the mature South African ART program, undisclosed ART use is common at 20% but is not associated with a reduced risk of undiagnosed TB. Undisclosed ART use may result in inappropriate use of resources and baseline testing. Use of a point-of-care urine assay to assess TFV could improve costly CD4 testing, however TB testing among all ART users should continue.

### 1203 Impact of Proactive Adherence Interventions on HIV RNA Use:

**Prescription Claims**

**Neha S. Pandit,** Abree Johnson, Tsung-Ying Lee, Eberuchukwu Onukwugha, Hope Cassidy-Stewart

1University of Maryland, Baltimore, MD, USA, 2Maryland Department of Health and Mental Hygiene, Baltimore, MD, USA

**Background:** Adherence interventions are often implemented after virologic failure is identified. Antiretroviral therapy (ART) prescription claims data can guide adherence interventions for people with HIV (PWH) at risk of virologic failure. This study determined the effectiveness of using ART prescription claims to provide adherence interventions and achieve virologic suppression (VS).

**Methods:** Participants were deemed eligible if they: 1) received care at a collaborating clinic; 2) failed to pick up ART at least 30, 60, and 90 days from prior fill date; 3) eligible to receive an intervention between November 2020 and December 2021; and 4) had an HIV RNA 12 months prior to and following the index date (date of intervention). Interventions were categorized as “no” (no patient contact), “soft” (indirect patient contact: e.g., voicemail), or “full” (direct patient contact: e.g., in-person visit). Across intervention groups, mean difference in HIV RNA was calculated and proportion of patients with VS (HIV RNA <200 copies/mL) was reported. We compared the pre- and post-index VS proportions by intervention group and reported differences using the Chi-square test with an a priori significance level of 0.05.

<table>
<thead>
<tr>
<th>Characteristics of PWH</th>
<th>n (%)</th>
<th>Adjusted OR (95% CI) for Undisclosed ART</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: Male vs. Female</td>
<td>128/288 (45%)</td>
<td>0.78 (0.60-1.04)</td>
<td>0.08</td>
</tr>
<tr>
<td>Age: 35-49 (vs. 18-29 ref)</td>
<td>146/170 (86%)</td>
<td>4.19 (2.02-8.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4: &lt;350 vs. 350-500 (ref)</td>
<td>176/151 (11%)</td>
<td>0.59 (0.36-1.02)</td>
<td>0.45</td>
</tr>
<tr>
<td>500-999 (vs. 500-999 ref)</td>
<td>85/85 (51%)</td>
<td>1.57 (0.54-4.91)</td>
<td>0.36</td>
</tr>
<tr>
<td>Clinical Initiation: Urban vs. Rural (ref)</td>
<td>200/235 (86%)</td>
<td>1.03 (0.79-1.32)</td>
<td>0.91</td>
</tr>
</tbody>
</table>

**Conclusion:** In the mature South African ART program, undisclosed ART use is common at 20% but is not associated with a reduced risk of undiagnosed TB. Undisclosed ART use may result in inappropriate use of resources and baseline testing. Use of a point-of-care urine assay to assess TFV could improve costly CD4 testing, however TB testing among all ART users should continue.

### 1204 Antiretroviral Therapy Outcomes at 6 Months by HIV Recent Infection Classification, Rwanda 2021-2022

**Monita R. Patel,** Eugenie Poirier, Straso Jovanovski, Beata Sangwayire, Jean Claude Irabona, Veronica Mugisha, Collins Kamanzi, Eric Remera, Elysee Tuyishime, Giles A. Reid, Tom Olouch, Suzue Saito, Gallician Rwibasa,

1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2ICAP at Columbia University, New York, NY, USA, 3Centers for Disease Control and Prevention, Kigali, Rwanda, 4ICAP at Columbia University, Kigali, Rwanda, 5Rwanda Biomedical Centre, Kigali, Rwanda, Rutgers University, New York, NY, USA

**Background:** Globally, over 25 countries have implemented HIV-1 infection surveillance to help detect and monitor recent infections among those newly diagnosed. As countries approach epidemic control and focus on early detection and life-long effective antiretroviral therapy (ART), we expect that an increasing proportion of persons newly diagnosed with HIV may be recently infected. However, there is limited evidence on whether persons with recent infection are more or less likely to initiate and stay on effective ART compared to persons with long-term infection.

**Methods:** We analyzed longitudinal data on newly diagnosed adults (>15 years old) enrolled in a cohort study conducted across 60 facilities in Rwanda from August 2021 to October 2022. Per study procedures, all participants received both rapid testing for recent infection (RTRI) and viral load at baseline and were classified as recent (RTRI-negative and baseline viral load >1000 copies/mL) or long-term (RTRI-negative and baseline viral load >1000); participants with baseline viral load <1000 were presumed previously diagnosed and an ART and excluded from analyses. Demographic and clinical HIV data were abstracted from Rwanda’s case-based surveillance system at baseline, and monthly through 6 months follow-up. We compared 6-month ART retention and viral suppression outcomes by recent status using Fisher’s exact and Wilcoxon rank tests.

**Results:** A total of 1,238 newly diagnosed persons were identified from the study. Overall, 99.4% (n=1,231) of clients initiated ART. Of these, 8.0% (n=98) were classified as recent. ART initiation was same-day on average (median=0 days [interquartile range: 0-1 days]), and did not differ by recent status (p=0.159). Among the 98.5% (n=1,219) of persons who initiated ART and had longitudinal data available, 78.2% (953) persons were retained on ART at 6 months, and retention did not differ by recent status (p=0.618). Among those retained on ART, 86.1% (n=821) were virologically suppressed at 6 months (<1000 copies/mL), and viral suppression did not differ by recent status (p=0.766).

**Conclusion:** Our findings suggest that ART initiation and 6-month longitudinal outcomes do not differ between clients classified as recent or long-term. Regardless of population trends in recent infections among persons newly diagnosed over time, programmatic efforts to continue to ensure timely ART initiation and retention will be needed.

### 1205 Impact of Natural Disasters on HIV Risks and Viral Suppression in Ugandan Fishing Village

Hadija Nakawooza, Victor Ssempija, Anthony Ndyabanjo, Fred Nalugoda, Maria J. Waver, David Serwadda, Ronald H. Gray, Joseph Kaggwa, Steven J. Reynolds, Tom Lutalo, Godfrey Kigozi, Ping T. Teh, Larry W. Chang, Mary Kate Grabowski, Robert Sokubugu

1Kakiri Health Sciences Program, Kakiri, Uganda, 2Lesbou Medical Research, Inc, Frederick, MD, USA

**Background:** Understanding the impact of natural disasters on the HIV epidemic in populations with a high HIV burden is critical for effective control efforts. We assessed HIV outcomes in a high-HIV prevalence Lake Victoria fishing community before and after COVID-19 pandemic and a severe lake flooding event in 2020.

**Methods:** We used data from the largest Lake Victoria fishing community in the Rakai Community Cohort Study, a population-based HIV surveillance cohort in south-central Uganda, collected prior to (September-December 2018) and after (October-December 2021) COVID-19 pandemic and a severe flooding event, to evaluate the impact of natural disasters on population-level HIV outcomes including HIV risk behaviors, seroconversion, and viral suppression among people living with HIV. Households impacted by flooding were identified using drone images and through consultation with community health workers. Differences in HIV-related outcomes before and after flooding/COVID-19, and...
between residents of flooded and non-flooded households were assessed using difference-in-difference statistical modeling.

**Results:** 1,226 people participated in the pre- and post-COVID surveys, of whom 506 (41%) were affected by flooding and 513 (41.8%) were female. HIV seroprevalence pre-COVID was 37% in both flooded and non-flooded households. Following onset of the COVID-19 pandemic, there was a decline in HIV risk behaviors. Transactional sex declined from 29.4% to 24.8% (p=0.011), inconsistent condom use with non-marital partners declined from 41.6% to 37% (p=0.021) in the pre- and post-COVID periods. ART coverage significantly increased from 91.6% to 97.2% (p<0.001). There was also a non-statistically significant increase in HIV viral load suppression in both flooded and in non-flooded households following COVID-19.

**Conclusion:** Despite a high background HIV burden, the COVID-19 pandemic and serious flooding, had no adverse impact on key HIV risk and outcomes. This may be attributable to innovative programming and or population resilience. Understanding what HIV strategies helped maintain good public health outcomes despite extreme conditions may help improve HIV epidemic control during future natural disasters.

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### 1206 Prevalence of HIV and Viral Load Suppression Among Refugees in Uganda, October to December 2021

**Authors:** Samuel Biraro, Shannon M. Farley, Joshua Musinguzi, Wiford Kirungi, David Okimait, Veronica Mugisha, Ronald Nakyojojo, Sam Sendagala, Herbert Kiyungi, Jennifer Nel, Lisa Nelson, Brittany Gianetti, Christine West, David Hoots, Wafaa El-Sadr

**Methods:** Using a multistage probability sampling design, 11 settlements, 40 enumeration areas and 1,296 households were selected. All adults (>15 years) in a selected household were eligible to complete the survey, and venous blood samples were tested for HIV, CD4 + cell count, viral load (VL) and presence of antiretrovirals (ARVs). VLS estimation included all PWH, irrespective of their antiretroviral therapy (ART) status.

**Results:** Overall, 93.4% of 1,250 eligible households, and 84.2% of 2,999 eligible adults (87.3% women, 79.5% men) participated. Most participants (44.7%) were aged 15-24 years; 61.6% reported living in the settlements for 3 to 5 years at the time of the survey; and the most common countries of origin were South Sudan (61.4%) and the Democratic Republic of Congo (31.9%). The prevalence of HIV among adults aged >15 years was 1.5% (95% CI: 0.8-2.1); 1.8% (95% CI: 1.0-2.7) among women and 1.1% (95% CI: 0.3-1.8) among men. HIV prevalence was highest among women aged 45-49 years at 6.2% and men aged 50-54 years at 5.8%. The prevalence of VLS among all PWH, irrespective of their awareness of their HIV status, was 72.4% (95% CI: 59.8-85.1) among those aged ≥15 years. Among PLHIV ≥15 years, 81.8% were aware of their HIV status, 89.5% of those aware of their status were on antiretroviral treatment (ART), and among those on ART, 92.5% had VLS.

**Conclusion:** In this refugee population, overall HIV prevalence was at 1.5%; however, prevalence among older women and men was above 5%. Overall, one out of five PWH was not aware of their HIV status and overall VLS was low at 72.4% indicating that more than a quarter of PWH had unsuppressed VL. These findings suggest that a substantial number of PWH in this refugee population would benefit from expansion of tailored case-finding and treatment support efforts.

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### 1207 COVID-19–Associated ART Disruptions and HIV Suppression: A Population-Based Study in Uganda

**Authors:** Charles Ssuuna, Hadjija Nakawooya, Caitlin E. Kennedy, Joseph G. Rosen, Ronald M. Galiwango, Aggrey Anok, Fred Nalugoda, Arthur G. Fitzmaurice, Victor Sempijja, Joseph Kagayi, Godfrey Kigozi, Larry W. Chang, Thomas C. Quinn, Mary Kate Grabowksi, Steven J. Reynolds

1. Rakai Health Sciences Program, Kalisizo, Uganda
2. The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
3. Centers for Disease Control and Prevention, Kampala, Uganda
4. Lexos Biomedical Research, Inc, Fredrick, MD, USA
5. The Johns Hopkins University, Kalisizo, Uganda
6. National Institute of Allergy and Infectious Diseases, Baltimore, MD, USA

**Methods:** We used cross-sectional data collected between October 2020 and March 2023 from the Rakai Community Cohort Study (RCCS), a population-based HIV surveillance cohort in south-central Uganda, to assess occurrence of COVID-19-related ART disruptions and their impacts on viral load suppression (VLS) among people living with HIV in Uganda.

**Results:** Overall, 2,634 of 2,786 (94.5%) people living with HIV self-reported being on ART, of whom 4.8% (n=126) and 13.5% (n=355) respectively reported ART treatment disruptions prior to COVID-19 emergence and 13.5% (n=355) following it. All ART disruption types increased significantly following COVID-19 emergence: missed HIV care appointments (3.3% to 9.8%, p<0.001), running out of ART before the next refill (2.3% to 5.4%, p<0.001), and reducing ART use to conserve medication supply (1.1% to 2.5%, p<0.001). Proportions of participants reporting ART disruptions before and after the lockdown were compared using chi-squared tests. We used modified Poisson regression models to estimate prevalence ratios for two outcomes: COVID-19-associated ART disruptions and VLS.

**Conclusion:** ART disruptions, especially missed HIV care appointments, increased significantly following COVID-19 emergence in Uganda. VLS was significantly lower among individuals who experienced COVID-19-related ART disruptions. Developing interventional effects in maintaining HIV patients in care is crucial to mitigate treatment disruptions during future pandemics.
1210 COVID-19 Vaccine Effectiveness Against Different Variants Among a Population-Based HIV Cohort

Ziang Liu, Xueying Yang, Bankole Olutosi, Sharon Weissman, Xiaoming Li, Jiagia Zhang
University of South Carolina at Columbia, Columbia, SC, USA

Background: COVID-19 vaccine effectiveness among people with HIV (PWH) was understudied as they were not representatively included in clinical trials. Using a test-negative design, we estimated vaccine effectiveness (VE) against the SARS-CoV-2 infection in different periods of circulating variants of concern (VOC) among a statewide cohort of PWH in South Carolina (SC), USA.

Methods: A population-based cohort was retrieved from the integrated statewide HIV electronic health record (EHR) data up to December 31, 2020 in SC. The adult PWH dataset was linked to COVID-19 vaccination dataset with record from January 1, 2021 and June 14, 2022 to identify the vaccination status. Outcome was any SARS-CoV-2 infection. Then we compared the odds of vaccination between test-positive cases and test-negative controls using logistic regression models in different period of circulating VOC, adjusting for include age, sex, race/ethnicity, and number of comorbidities (Charlson Comorbidity Score [CCI]). VE was derived as (1-adjusted odds ratio)×100%.

Results: Among a total of 6,239 PWH, 2,180 were test-positive cases and 4,059 were test negative controls. Among them, 48.3% aged 50 years and above, 68.7% were male, 71.2% were black. The coverage rate of fully vaccination rate was similar between cases (33.0%) and controls (32.0%), while a higher coverage of booster dose (8.3%) was observed among cases than controls (7.0%). When stratified by different VOC, VE was 61.47% and 79.31% for partial and fully vaccination status in the Alpha dominant period. In the Delta dominant period, VE was 13.88%, 37.46%, and 69.17% for partial, fully, and booster vaccination status. In the Omicron dominant period, the VE was only 22.20% for individuals who received booster dose. When look at the VE in different vaccine brands, BNT162b2 indicated a higher VE during Alpha period, mRNA-1273 revealed a high VE (54.26%) during Delta period. In contrast, Ad26.COV2.S showed the higher VE during Omicron period.

Conclusion: The VE against SARS-CoV-2 infection among PWH substantially decreased during Omicron dominant period compared with the other periods. Fully vaccination, especially boosted vaccination, offered sustained protection prior to the emergency of the Omicron VOC. In terms of vaccine brands, our findings suggested that both BNT162b2 and Ad26.COV2.S had lasting protection effect prior to the Omicron period while the protection effect of mRNA-1273 gradually diminished along with periods.
1212 Safety Outcomes in Trial of COVID-19 mRNA Vaccine Among People With HIV in Sub-Saharan Africa
Simone L. Hendricks, Bongile Mbilane, Asa Tapley, Jia Jin Kee, Veronique Bailey, Ethel Kamuti, Harriet Nwagaba-Biribonwoh, Zvavahera Chirenje, Joseph M. Makhema, Taraz Samandari, Laura Polakowski, Nyaradzo Mpofu, Azridwihlu Takalani, for the CoVPN 3008 Study Team

Background: The study enrolled adults age ≥ 18 years living with HIV or another chronic infection. Participants were assigned vaccinations based on their baseline serostatus: those with a positive result received one dose at baseline and month 1 if asymptomatic; those with a negative result received a dose at baseline and month 1 if symptomatic. Each of the other intervention strategies produced a PIA > 10% independently, with the 13% jail release scenario producing a PIA of nearly 20%. The all-level contact tracing only scenario was effective at both 50% and 100% of contacts traced, but feasibility is limited without a reduction in the jail population.

Conclusion: Implementing combined interventions could substantially reduce the morbidity and mortality from COVID-19 and future airborne pathogens in a jail setting while providing secondary protection to the community.

1213 Data Science Linkage of Public Jail Registries and Surveillance Data for Public Health Action
Steven Erly, Leighton Hill, Rachel Amiya
Washington State Department of Health, Olympia, WA, USA

Background: Jail bookings represent an important opportunity for diagnosis and care linkage for people living with HIV (PLWH). However, jurisdictional data protections and decentralization of facilities can make connecting public health resources to correctional programs challenging. The purpose of this study was to evaluate the use of a novel approach using data scraping of publicly available jail inmate data to develop a real-time dataset of public jail rosters and to estimate the number of PLWH who have regular interactions with jails in Washington State (WA).

Methods: We developed scripts to collect public-facing online jail rosters from local and tribal facilities in WA using R software. Between 11/14/2022 and 8/13/2023, we ran these scripts daily and developed a longitudinal database of the incarcerated population. We used a deterministic matching algorithm to link this database to the state HIV registry (as of 7/1/2023) using name, date of birth, and county of residence. We described the population that was jailed by demographic characteristics and CDC-defined viral suppression status on 12/31/2022. We used a zero-truncated Poisson model to estimate the total population of PLWH who are jailed on a regular basis and the subset of this population who were not virally suppressed.

Results: We successfully captured data from 57 of the 59 jails identified by the authors in WA. The remaining two jails were small county facilities which were not in compliance with state reporting requirements. In the 9-month study period, we collected 2,270,716 person-days of incarceration data representing 79,088 individuals. We identified 411 PLWH in this population who were not virally suppressed (33%, estimated population size: 253, 95% CI 212-294). 18.5% had tuberculosis, carcinoma, and homicide. Among 236 pregnancy outcomes reported, there were 23 premature live births, 12 spontaneous abortions, 9 still births, and 4 congenital anomalies. There were no Covid-19 deaths or admissions to the intensive care unit.

Conclusion: Severe Covid-19 was rare in this large, diverse population of PLWH during the Omicron wave. In SSA, the mRNA vaccine demonstrated an acceptable reactogenicity and safety profile, including in PWH, people with prior SARS-CoV-2 infection, and pregnant participants.

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1214  **Undetectable = Untransmitizable: Key Populations With HIV Lag in Sustaining Undetectable Viral Load**  
Abimbola S. Phillips1, Dorcas Magabedile1, Francis Ogrima2, Pius Christopher-Izere3, Collins Imaimahiagbe4, Sabra Custer5, Sharon Weisman5, Bankole Olatoji6, Xiaoming Li7, Oluwafemi Adeagbo8, Akamnu Salaiyin9, Akinbami Akinsegun9, Adekunbo O. Oyelude1, Jude Olifi10, Dennis Onout10, for the CDC Research Group

*Centre for Integrated Health Programs, Abuja, Nigeria, 1University of South Carolina at Columbia, Columbia, SC, USA, 2University of Iowa, Iowa City, IA, USA, 3LAGOS State University Teaching Hospital, Lagos, Nigeria, 4Centers for Disease Control and Prevention, Abuja, Nigeria*

**Background:** Undetectable viral load (UVL) is the optimal suppression level in people with HIV (PWH) required to maintain health, prevent transmission, and assure safer sex with partners. In Nigeria, about 80% of PWH achieve UVL on treatment. However, this must be sustained over the course of life-long anti-retroviral treatment (ART) for continued benefits. We compared durable UVL levels among key population (KP) groups — female sex workers (FSW), men having sex with men (MSM), and people who inject drugs (PWID) — and the general population (GP).

**Methods:** A cross-sectional retrospective study of PWH diagnosed from 2020 to 2021 using data retrieved from electronic medical records in 57 health facilities across four states (Gombe, Kaduna, Kogi, and Lagos) in Nigeria. We defined durable UVL as viral load of <50 copies per ml from two tests conducted at least 6 months apart. We compared distributions between KP groups and GP using chi-squared tests. Multivariate logistic regression with robust variance estimation was used to obtain adjusted odds ratio (AOR) with 95% confidence interval for durable UVL and other variables in these categories.

**Results:** Within this period, 6,680 client records were reviewed and 5,286 (79%) had achieved UVL within 6 months of ART. This was significantly higher amongst KP (92% [329/358] than GP (78% [4,957/63,221]) \( (p < 0.001) \). Among those with UVL, the median age was 33 years (IQR 28 − 40), 65% were females, 59% attained at least secondary education, 77% were unemployed, 62% were married, and 87% received care in their state of residence. Four thousand, three hundred and forty-five clients (82%) had durable UVL, and this was higher among KP (84%) than amongst GP (FSW − 38%; MSM − 50%; PWID − 61%). \( (p = 0.001) \). Multivariate analysis showed lower odds of durable UVL among KP groups compared to GP (FSW: AOR = 0.16 [0.12 − 0.20]; MSM: AOR = 0.25 [0.20 − 0.32]; PWID: AOR = 0.41 [0.31 − 0.53]). The odds of durable UVL were lower among the employed than unemployed (AOR = 0.63 [0.45 − 0.89]), and higher among clients aged 40 − 44 years than those 15 − 19 years old (AOR = 1.70 [1.03 − 2.80]).

**Conclusion:** These findings from these four states suggest that although KP groups appear to be more likely than GP to achieve early UVL, they are less likely to sustain it. Providing insights for these differences will help develop appropriate interventions to achieve and sustain viral suppression amongst clients with HIV on ART irrespective of subpopulation.

1215 **PrEP Persistence Among Adolescents Men Who Have Sex With Men and Transgender Women in Brazil**

Fabiane Soares1, Laino Magnô1, Jony Arrais Pinto Junior1, Diana Reyna Zeballos Rivas1, Dirceu Greco1, Alexandre Grangeiro2, Jony Arrais Pinto Junior1, Ines Bourado3,4,5

1Federal University of Bahia, Salvador, Brazil, 2Bahia State University, Salvador, Brazil, 3Universidade Federal Fluminense, Rio de Janeiro, Brazil, 4Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, 5University of Sao Paulo, Sao Paulo, Brazil

**Background:** Adolescent men who have sex with men (AMSM) and transgender women (ATGW) are at a heightened vulnerability for HIV in Brazil. Promoting the use and persistence of HIV prevention strategies, including Pre-exposure Prophylaxis (PrEP), is a relevant challenge for controlling the epidemic in these populations. We aim to analyze the persistence of daily oral PrEP among AMSM and ATGW and their associated factors.

**Methods:** The PrEP1519 study is a daily oral PrEP demonstration cohort study with AMSM and ATGW aged 15 to 19 years, implemented in three large capital cities in Brazil: Salvador, Belo Horizonte and São Paulo. We included in this analysis participants who initiated PrEP between Feb 2019 and Feb 2021 and were followed up until Feb 2022. Study visits occurred at baseline, 30 days, 60 days and quarterly thereafter. PrEP persistence was defined as being in PrEP possession in Feb/2022 or having less than 90 days without PrEP possession. Logistic regressions were used to estimate the odds ratios (OR) of sociodemographic and behavioral factors with persistence in PrEP use.

**Results:** In the first two years of the cohort, 735 adolescents initiated daily oral PrEP. The majority of them was between 18 and 19 years old (79.5%), were MSM (91.1%), black and brown (70.1%), and had 12 years of schooling or more (50.8%). 330 (43.7%) adolescents persisted on PrEP, their mean followed time was 18.7 months (95% CI 17.9% − 19.4%). Adolescents who persisted on PrEP use had more chance to be black or mixed race (OR: 1.49; IC 1.08 − 2.06) and with moderate and high adherence to self-report (OR 3.07: IC 1.99 − 4.77). No association was observed between PrEP persistence with sexual behavior and HIV risk perception.

**Conclusion:** The persistence of daily oral PrEP use was observed in approximately half of the adolescents, those who had higher PrEP adherence. Our results emphasize the need for strategies to support PrEP persistence in the clinics. Besides, other alternatives for prevention, such as PrEP on-demand and long-acting injectable, may be more attracted and fit better with adolescents, considering their experiences and preferences.

1216 **NICE: A Structural Intervention to Increase Status Neutral Care Among Sexual and Gender Minorities**

Rebecca Eavou1, Elin Almirol1, Victoria Umutoni2, John Schneider3, Mickyala Jones3

1University of Chicago, Chicago, IL, USA, 2Centers for Disease Control and Prevention, Abuja, Nigeria

**Background:** Black and Hispanic Sexual and Gender Minorities (SGM) are highly impacted by HIV. Significant structural determinants of health, such as access to care, are major drivers of status neutral care engagement, an ending the epidemic imperative. We designed an insurance navigation intervention – NICE (Navigating Insurance Coverage Expansion) – that tested the impact of navigation at the point of community HIV testing and evaluated the effects of the intervention on successful insurance enrollment and linkage to status neutral care among SGMs.

**Methods:** NICE aimed to test whether providing in-person assistance (enrolling, changing or learning how to use health insurance), at the HIV testing event would improve linkage rates for participants, particularly among persons living with HIV (PLWH). Black/Hispanic SGM aged 18 or older and living in Chicago were enrolled at community outreach testing events and randomized to NICE versus standard of care. Logistic regression was performed to see if there were differences in HIV linkage, PrEP linkage, and/or both (status neutral) by intervention assignment.

**Results:** A total of 630 participants were enrolled, with 281 in the health insurance enrollment assistance arm and 349 in the control, with a third of the sample living with HIV (29.4%), trans* representing 8.1%, and a sizeable proportion insured at baseline (68.4%). There were no differences in sociodemographic factors at baseline across study conditions. Overall, about 46.7% PLWH were linked to care (OR 1.37, 95% CI 1.04−2.48; p=0.32), while only 16.5% for those HIV negative who attended PrEP providing clinic within 90 days (OR 0.84, 95% CI 0.50−1.43; p=0.53), however, not significant by intervention condition. Overall, about a quarter of participants were considered linked (25.8%) and there were no differences in status neutral outcomes across conditions when analysis was limited to the uninsured.

**Conclusion:** Health insurance navigation, while likely an important component in accessing status neutral care, did not significantly impact linkage to care. Overall, linkage was low among SGM who had high rates of insurance at baseline. Future research should examine SGM understanding of their insurance conditions when analysis was limited to the uninsured.

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**Methods:** Using the Reach, Effectiveness, Adoption, Implementation, and Maintenance (RE-AIM) implementation science framework, we assessed DREAMS implementation from 2016 to 2022 in 14 Zambian districts. We used a mixed-methods approach including aggregate and line-listed client-level data for quantitative analyses across programs, costing analyses, and a three-site qualitative case-study with 134 interviews and 6 focus group discussions with beneficiaries, implementers, and stakeholders. Data were analyzed using R-Studio and Atlas.ti.

**Results:** From October 2016 to September 2022, 1,091,641 AGYW were enrolled, with 976,689 accessing DREAMS services. Among enrolled AGYW, 350,127 (32.1%) were 10-14 years, 441,402 (40.2%) were 15-19 years, and 295,291 (27.1%) were 20-24 years. Engagement in the primary package of social asset building was high with 97.3% (952,947) of enrolled AGYW completing all 13 Stepping Stones sessions. Socioeconomic support services were the most commonly accessed DREAMS secondary service with 29.6% participation, while biomedical services, including condom distribution, family planning, and HIV testing, followed closely with 27.2%, 11.2%, and 9.6% participation, respectively. Overall, 2,328 (2.2%) of 104,859 AGYW tested positive for HIV. Qualitative data revealed high levels of satisfaction with the program, especially concerning the primary intervention package. Beneficiaries reported that the program enabled them to adopt condom use, increased their awareness of PrEP benefits, and enhanced their assertiveness and control over their sexual health and decision-making within intimate relationships. However, qualitative findings indicated need for further measures to improve the program’s capacity to reach AGYW most at-risk of HIV acquisition.

**Conclusion:** DREAMS successfully reached large numbers of at-risk AGYW in Zambia with HIV services, demonstrating potential effectiveness, high adoption rates, and effective implementation. Maintenance efforts are ongoing and yet to be determined. Holistic prevention programs like DREAMS should be considered for further scale-up of HIV prevention.

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**1218 Intention to Initiate PrEP Among Men Who Have Sex With Men (MSM) and Transgender Women (TGW) in Peru**

Carlos Benites1, Mary Reyes1, Patricia Alarcón2, Ricardo Alfaró1, Jorge A. Gallardo-Cartagena1, Juan J. Montenegro-Idrogo1, Kelika A. Konda3, Jorge L. Sánchez4, César Munayoa1

1Ministry of Health, Lima, Peru, 2Asociacion Civil Impacta Salud y Educacion, Lima, Peru, 3Universidad Nacional Mayor de San Marcos, Lima, Peru, 4Other Institution – Follow-up needed, Callao, Peru, 5University of Southern California, Los Angeles, CA, USA

**Background:** Recently, Peru incorporated PrEP into their combination HIV prevention strategy. However, data from the ImPrEP demonstration project shows that PrEP adherence and persistence are problematic among Peruvian MSM/TGW. As national scale-up of PrEP has begun, reasons behind poor progression along the PrEP cascade need to be fully understood. We aimed to explore factors associated with the intention to initiate PrEP among Peruvian MSM/TGW using national surveillance data.

**Methods:** Between April and July 2019, the HIV/STI Sentinel Surveillance was conducted in 11 Peruvian cities, enrolling 1768 MSM and 1198 TGW aged 18+ who provided informed consent. The survey collected socio-demographics, sexual behavior, self-reported STI diagnosis, and HIV testing history, PrEP awareness, and intention to initiate PrEP, along with sample collection for HIV/STI testing. Respondents who self-reported as HIV negative and had not initiated PrEP were included in the analysis. We used Poisson regression to identify factors associated with the intention to initiate daily oral PrEP, using covariates selected a priori, and stratifying by population.

**Results:** The analysis included 1582 MSM and 904 TGW. Median age was 25 yo (IQR 21-32) among MSM and 28 (IQR 23-37) among TGW. Intention to initiate PrEP was 48.9% among MSM and 60.7% among TGW. Other covariates included in the analysis included monthly income (MSM: median US$2543 [IQR 162-324]), TGW: median US$3243 [IQR 135-324]), post-secondary education (MSM 36.5%, TGW 21.0%), prior PrEP awareness (MSM 16.1%, TGW 23.3%), having ≥5 sex partners in the past 6 months (MSM 30.9%, TGW 63.6%), engagement in transactional sex (MSM 24.6%, TGW 54.5%), prior HIV testing (MSM 66.4%, TGW 80.0%), and self-reported STI diagnosis (MSM 12.0%, TGW 23.4%). Age, post-secondary education, prior HIV testing remaining, and engagement in transactional sex, were significant covariates for intention to initiate PrEP among MSM; while prior PrEP awareness and engagement in transactional sex were significant covariates for TGW (Table 1).

**Conclusion:** Factors significantly associated with intention to initiate PrEP varied by population, though engagement in transactional sex was associated with intention to initiate PrEP in both groups. In the context of national PrEP scale-up, these findings could inform differentiated interventions to improve PrEP uptake among MSM/TGW. Issues of PrEP awareness and steps along the PrEP cascade toward planning and uptake should be addressed.

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**1219 WITHDRAWN**

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**1220 The Motivational Pre-Exposure Prophylaxis Cascade Among Peruvian Men Who Have Sex With Men (MSM)**

Jorge A. Gallardo-Cartagena1, Patricia Alarcón1, Juan J. Montenegro-Idrogo1, Javier R. Lama2, Javier A. Valencia3, Robinson Caballo1, Martin Casapia1, David Velasquez1, Hugo Sánchez1, Yamir Salazar1, Felipe Vilchachagua Tadeo1, José L. Castro1, Kelika A. Konda2, Susan F. Buchbinder3, Jorge L. Sánchez3, Kinley M. Samwel4, Edward Mboya5, Violet Booko1, Peter Kyalo1, Kinley M. Samwel, Edward Mboya, Violet Booko, Peter Kyalo

1Universidad Nacional Mayor de San Marcos, Lima, Peru, 2Asociación Civil Impacta Salud y Educación, Lima, Peru, 3University of Southern California, Los Angeles, CA, USA, 4San Francisco Department of Public Health, San Francisco, CA, USA

**Background:** In several recent studies, MSM HIV incidence was >5/100 person-years in Peru, where PrEP scale-up is urgently needed. The Motivational Pre-Exposure Prophylaxis (MPC) explains dynamic movement on a PrEP continuum including
planning, uptake, and persistence. We aimed to describe key MPC steps and interest in tailored mHealth interventions among Peruvian MSM, at the time the National PrEP Program (NPP) began in mid-2023.

Methods: We conducted an online survey from June to August 2023. MSM were recruited through social media ads. Following informed consent, respondents answered a survey on demographics, sexual behavior, key MPC steps, smartphone use, and mHealth intervention preferences. Only PrEP candidates based on NPP guidelines (HIV negative by self-report with condomless anal sex in past 6 months) were included in this analysis. We describe the distribution of DSM by MPC steps and identified associated factors using multivariate Poisson regression.

Results: Among the 464 included respondents, median age was 29yo (IQR: 25-35). Most respondents earned Conclusion: Peruvian MSM who completed a PrEP survey when the NPP began showed great interest in PrEP. Self-identified as good PrEP candidates, and/or decided to start PrEP. However, most MSM struggled to progress to later MPC steps, highlighting access barriers outside of research studies. The MPC could help identify subgroups with unique informational and/or skill development needs, which can be used in the design of tailored mHealth interventions supporting the NPP implementation in Peru.

<table>
<thead>
<tr>
<th>Table 1: Associations of selected covariates* with key MPC steps (adjusted prevalence ratio with 95% CI)**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PrEP candidates</strong></td>
</tr>
<tr>
<td><strong>Post-secondary education (57.3%)</strong></td>
</tr>
<tr>
<td><strong>Income (28.0%)</strong></td>
</tr>
<tr>
<td><strong>Utilization of care (53.2%)</strong></td>
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<tr>
<td><strong>Stigma</strong></td>
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<tr>
<td><strong>HIV Risk Factors</strong></td>
</tr>
<tr>
<td><strong>Have co-occurring health concerns (59.6%)</strong></td>
</tr>
</tbody>
</table>

1. Each model additionally adjusted for: city, STI diagnosis, transactional sex, condom use, and partner HIV status. *Unadjusted p<0.05

1221 The Need to Address Violence for PrEP Uptake Among Adolescent Girls and Young Women in South Africa

Courtney P. Bonner, Felicia A. Browne, Jaceque U. Nidrangi, Alexandra M. Minnis, Ilene Speizer, Laura Hyblade, Khatija Ahmed, Tracy Kline, Wendee M. Wechsberg

RTI International, Atlanta, GA, USA; RTI International, Research Triangle Park, NC, USA; RTI International, Berkeley, CA, USA; University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; RTI International, Washington, DC, USA; 1st Referral Centre for Research, Thohle, South Africa

Background: Adolescent girls and young women (AGYW) account for 25% of new HIV infections in South Africa. We have conducted several NIH-funded studies with AGYW to address HIV risk and prevention and gender-based violence, yet pre-exposure prophylaxis (PrEP) had not been approved. Working with the national government as PrEP was approved and rolled out in South Africa, we conducted an online survey from June to August 2023. MSM were recruited through social media ads. Following informed consent, respondents answered a survey on demographics, sexual behavior, key MPC steps, smartphone use, and mHealth intervention preferences. Only PrEP candidates based on NPP guidelines (HIV negative by self-report with condomless anal sex in past 6 months) were included in this analysis. We describe the distribution of DSM by MPC steps and identified associated factors using multivariate Poisson regression.

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A Social Network-Based Intervention to Promote HIV Prevention and Treatment Among Fishermen in Kenya

Zachary A. Kweka, Lila Sheira, Benard Ayiekoi, Edwin Charlebois, Kawango Agot, Sarah Gutin, Phoebe Gluglo, Monica Gandhi, Elizabeth Bukusi, Carol S. Camlin, Harsha Thirumurthy

1Kenya Medical Research Institute, Kisumu, Kenya, 2University of California San Francisco, San Francisco, CA, USA, 3Impact Research and Development Organization, Kisumu, Kenya, 4Medical Research Institute, Nairobi, Kenya, 5University of Pennsylvania, Philadelphia, PA, USA

Background: Men in sub-Saharan Africa are less likely than women to know their HIV status and utilize HIV prevention and treatment services. We previously showed a social-network-based intervention increased HIV testing uptake by 50% among men in Kenya. Here, we evaluate the impact of the intervention on HIV prevention and treatment outcomes using objective metrics.

Methods: Data are from the Owete study (NCT04772469), a RCT of an HIV status-neutral, social-network-based intervention to promote HIV self-testing and linkage to prevention and treatment among men in Lake Victoria fishing communities. After a census of fishermen, distinct social networks with a network-centric “promoter” were mapped and randomized to study arms. Promoters were asked to (1) distribute self-tests to men in their network and encourage linkage and retention in prevention and care (intervention clusters) or (2) distribute vouchers for free self-tests redeemable at study-affiliated health facilities (control clusters). We evaluated PrEP adherence measured via urine assay for tenofovir among men initiating PrEP, and HIV RNA viral load assessed via the Xpert assay (40 copies/ml threshold) among people with HIV (PWH), at 3 months. We coded missing viral load as failure (detectable). We conducted logistic regression controlling for site (beach) and with a random intercept for cluster to evaluate the intervention’s impact on PrEP adherence and viral suppression.

Results: Of 934 men in the intent-to-treat sample, 733 were interviewed at baseline (374 intervention) and 339 linked to study-affiliated clinics: 71 initiated PrEP, and 169 were PWH. Urine tenofovir was detected among 12 of 71 participants on PrEP (14% of control vs. 12% intervention), and 107 of 169 participants on ART had undetectable viral loads (58% of control vs. 69% intervention). We did not detect a statistically significant difference between study arms in PrEP adherence (odds ratio [OR]: 0.85; 95% CI: 0.17, 4.23, p = 0.84) or viral suppression (OR = 0.59; 95% CI: 0.29, 1.22; p = 0.16).

Conclusion: A social network-based, status-neutral intervention in Kenya that successfully promoted testing among men did not impact PrEP adherence or viral suppression, although we demonstrate preliminary indications of intervention effect at a relaxed alpha of 0.2. Given the small number of men on PrEP and ART, an adequately powered study is required to evaluate whether social-network-based interventions can improve these outcomes among fishermen and other hard-to-reach populations.

Homelessness and Antiretroviral Use Among MSM in the Context of Varying Levels of Federal Funding

Amrita Rao, Yungui Mi, Katherine Ruscinik, John Mark Wignorton, Carrie Lyons, Kalai Willis, Tiara Willie, Travis H. Sanchez, Stefan Baral

1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2University of California San Diego, La Jolla, CA, USA, 3Emory University, Atlanta, GA, USA

Background: Homelessness is associated with poor health outcomes among people living with HIV (PLHIV); gay men and other men who have sex with men (MSM) account for over 50% of PLHIV in the United States and over 70% of new diagnoses. The Housing Opportunities for Persons With AIDS (HOPWA) Program is operated by HUD to fund initiatives addressing the housing needs of PLHIV. There is limited research examining the relationship between homelessness and HIV-related outcomes in varying policy and funding contexts.

Methods: 3,636 cisgender MSM who participated from 2017–2021 in the American Men’s Internet Survey (AMIS) and self-reported a prior HIV diagnosis were included in analyses. Homelessness in the survey was defined as living on the street, in a shelter, in a Single Room Occupancy hotel, or in a car in the past 12 months. We used a multilevel mixed-effects logistic regression model to assess the association between homelessness and current antiretroviral therapy (ART) use, adjusting for age, race, education, recruitment year, injection drug use, and state-level Ryan White Part B funding. Analyses were stratified by state-level HOPWA funding per person: state-level HOPWA funding was divided by the number of sheltered and unsheltered people experiencing homelessness (Point-in-Time Count) multiplied by the 2021 HIV prevalence for each state.

Results: Overall, experiencing homelessness was associated with decreased likelihood of current ART use (aOR: 0.72, 95% CI [0.51, 0.99]). Among states who received at or below the median level of HOPWA funding per person (~$300K), homelessness remained associated with ART use (aOR: 0.49, 95% CI [0.27, 0.89]), while there was no association among states who received above the median (aOR:0.72, 95% CI [0.26, 2.00]), suggesting effect measure modification by funding level.

Conclusion: Homelessness impedes ART use among MSM living with HIV. In states that received a greater level of per-person funding, this relationship was attenuated, providing preliminary evidence that housing supports and interventions to address basic needs can have important impacts on community-level HIV service uptake and subsequent outcomes. Further research is needed to document the immediate and long-term impacts of this Program.

Methods: We conducted a pilot randomized trial of immediate fast-track vs. standard care among patients newly diagnosed with HIV in Port-au-Prince, Haiti. Patients were randomized in a 1:1 ratio to immediate fast-track care (expedited nurse-led visits with pre-packaged, point-of-care dispensing of ART) vs. standard care. The primary outcome was the proportion of patients with HIV-1 RNA <200 copies/ml at 48 weeks after enrollment. We also administered multiple questionnaires to assess predictors of viral suppression.

Results: From January 3, 2019 to April 15, 2020, 103 participants were enrolled in the trial (48 standard; 55 immediate fast-track). The median age was 38 years (IQR: 32, 47). 46.6% were female, and median CD4 count was 330 cells/mm$^3$ (IQR: 227, 520). In the standard group, 42 (87.5%) were retained, 2 (4.2%) died, and 4 (8.3%) were LTFU; 37/42 (88.1%) retained patients received 48-week viral load testing, and 32 had HIV-1 RNA <200 copies/ml (86.5% among those tested; 66.7% among those enrolled). In the immediate fast-track group, 52 (94.5%) were retained, 1 (1.8%) died, and 2 (3.6%) were LTFU; 44/52 (84.6%) retained patients received 48-week viral load testing, and 40 had HIV-1 RNA <200 copies/ml (90.9% among those tested; 72.7% among those enrolled).

There was no difference in the primary outcome between the two groups (p = 0.65). In multivariable analysis, the only predictor of virologic suppression was a higher score on the State Hope Scale, indicating a higher degree of hopefulness about the future.

Conclusion: Retention and viral suppression rates were high in both the immediate fast-track and standard groups. Hopefulness was the most important predictor of retention in care with viral suppression. Further study is necessary to determine if this finding is reproduced in other cohorts.

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1226 Violence and HIV Sexual Risk Behaviours Among Adolescent Girls and Young Women: Eswatini Experience

Siphiwe M. Shongwe-Gama,1 Ceboile Ngcamphalala,1 Mobutho C. Mamba,2 Harrison Kamiru,2 Poppity M. Sithole,2 Bonisile Nhlabatsi,3 Choice Gimҙında3, Michelle Li,4 Kaye Seya Marie4, Francis B. Amour,4 Lioua Chiang,5 Ruben Sahabo6
1ICAP at Columbia University, Mbabane, Eswatini, 2ICAP at Columbia University, New York, NY, USA, 3Deputy Prime Ministers Office, Mbabane, Eswatini, 4Ministry of Health, Mbabane, Eswatini, 5Central Statistical Office, Mbabane, Eswatini, 6US Centers for Disease Control and Prevention Mbabane, Mbabane, Eswatini, 7Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Experience of violence is reported as a precursor to multiple risk behaviors including HIV sexual risk behaviors (SRB). HIV SRB remains a public health concern as a possible cause of HIV, impacting progress towards achieving HIV epidemic control. However, there are limited contemporaneous national-level assessments of HIV prevalence, magnitude and impact of violence. We explored HIV SRB associated with history of violence among females ages 13-24 years in Eswatini.

Methods: Data from the 2012 Eswatini Violence Against Children and Youth Survey (VACS) was analyzed. VACS is a nationally representative household survey using a multi-stage sampling approach for males and females ages 13-24 years. Participants completed a questionnaire on demographics, sexual behaviors and practices, HIV/AIDS services history, and experience of any violence (physical, emotional, and sexual), and conducted HIV testing. The current study was limited to only females (N=6,318), aged 13-24 years. Using stepwise logistic regression with forward selection approach, we assessed the association between experience of any violence and HIV SRB. We controlled for HIV SRB: multiple sexual partners (two or more sexual partners in the past 12 months); infrequent condom; transactional sex; HIV status; history of STIs and age.

Results: The prevalence of any violence (sexual, physical or emotional) was 25.5% (95% CI: 23.3-27.7%) from the 6,318 females interviewed. In the logistic regression all the following variables were significantly associated with experience of any violence: transactional sex (AOR 2.7, 95% CI 2.0 – 3.7) compared to none; STI history (AOR 2.1, 95% CI 1.5 – 3.2) compared to those with no history of STIs; multiple sexual partners (AOR 1.7, 95% CI 1.2 – 2.3) compared to having one partner or none; infrequent condom use (AOR 2.0, 95% CI 1.5 – 2.6) and never using a condom (AOR 1.4, 95% CI 1.2 – 1.7) compared to always using a condom; age group of 13-17 years (AOR 1.5, 95% CI 1.1 – 1.9) compared to 18-24 years and HIV positive status (AOR 1.3, 95% CI 1.0 – 1.8).

Conclusion: Experience of any violence was associated with HIV SRBs among adolescent girls and young women. Targeted multi-pronged interventions including violence and HIV prevention, sexual reproductive health education services need to be intensified to address sexual risk behaviors among females in Eswatini.

1227 Older Adults Living With HIV Have Low Expectations Regarding Aging, Despite Improved Survival

Alice Zhabokritsky,1 Darrell H. Tan,2 Marianne Harris,3 Francis B. Annor1,2,4, Poppy M. Sithole5,6,7, Ron Rosenes8, Julian Falutz9, Maple Leaf Medical Clinic, Toronto, Canada, 1University of Toronto, Toronto, Canada, 2Center for the Study of HIV/AIDS, Vancouver, Canada, 3ICAP at Columbia University, Montreal, Canada, 4University Health Network, Toronto, Canada

Background: The life expectancy among people living with HIV approaches 90 years. Older adults living with HIV age 65 and older. Participants completed the Expectations Regarding Aging Survey (ERA-12) at cohort entry; subscale (physical health, mental health and cognitive function) and total scores were calculated on a scale of 0-100 with lower scores indicating lower expectations regarding aging. Multivariable linear regression was used to estimate the association between ERA-12 score, duration of HIV infection, and sociodemographic and clinical factors selected a priori (age, gender, race, depression, social support).

Methods: We performed a cross-sectional analysis of the Correlates of Healthy Aging in Geriatric HIV (CHANGE HIV) study, a Canadian cohort of people living with HIV age 65 and older. Participants completed the Expectations Regarding Aging Survey (ERA-12) at cohort entry; subscale (physical health, mental health and cognitive function) and total scores were calculated on a scale of 0-100 with lower scores indicating lower expectations regarding aging. Multivariable linear regression was used to estimate the association between ERA-12 score, duration of HIV infection, and sociodemographic and clinical factors selected a priori (age, gender, race, depression, social support).

Results: 320 participants were included in the analysis, of whom 91% identified as men and 78% as white, with a median (interquartile range [IQR]) age of 69 (67,73). The median [IQR] ERA-12 score was 47 (33,58). Expectations regarding mental health (median [IQR] score 58 [42, 83]) were higher than those for physical health (median [IQR] score 33 [17,50]) and cognitive function (median [IQR] score 42 [25,58]). In multivariable analysis, ERA-12 scores did not differ according to age, race, or duration of HIV infection. After accounting for other factors, women (β -9.87, 95% CI -18.39, -1.38, p =0.023), persons experiencing depression (β -15.96, 95% CI -22.34, -9.59, p <0.001) and those with greater degree of social isolation (β -0.05, 95% CI -0.22, -0.04, p=0.007) had lower expectations regarding aging.

Conclusion: Older adults living with HIV seem to have low expectations regarding their physical health and cognitive function, regardless of how long they’ve been living with HIV. Gender-specific differences in expectations persist after taking into account demographic factors, depression and lack of social supports. This reinforces the need to address physical and cognitive health concerns among persons aging with HIV.

1228 Strategies for Eliminating Racial/Ethnic Disparities in HIV Incidence in the United States

Evin Jacobson,1 Alex Viquezrie2, Katherine Hicks1, Laurel Bates1, Amanda Honeycutt1, Justin Carrico1, Paul Farnham3
1Centers for Disease Control and Prevention, Atlanta, GA, USA, 2RTI Health Solutions, Durham, NC, USA, 3RTI International, Research Triangle Park, NC, USA

Background: Elimination of racial/ethnic (r/e) disparities is a goal of the Ending the HIV Epidemic in the U.S. (EHE) initiative. Despite progress in HIV prevention and treatment, large r/e disparities in HIV incidence remain. We used the HIV Optimization and Prevention Economics (HOPE) model to analyze which interventions provide the most effective path towards eliminating r/e disparities in HIV incidence.

Methods: We considered a baseline Scenario A, which assumed continuation of HIV continuum of care and prevention efforts (pre-exposure prophylaxis (PrEP) and syringe services programs (SSPs)) at 2022 levels from 2023-2035. We considered these r/e groups: Black, Hispanic/Latino (H/L), and the remaining mostly White population grouped as Other. The primary outcome is the incidence-rate ratio (IRR) compared to Other, with the goal of IRR≤1 for both Black and H/L by 2035. We considered four intervention scenarios, B through E, by adjusting input values from 2023-2027, then we observed outcomes from 2027-2035: -Scenario B: Continuum- only - HIV testing, linkage to care and viral suppression among Black and H/L brought to parity with Other by 2027.-Scenario C: Prevention-only – PrEP and SSP uptake among Black and H/L brought to parity with Other by 2027.-Scenario D: Continuum+Prevent-Combined B and C-Scenario E: Max reach - Black and H/L populations reach 98% awareness, linkage to care, and viral suppression coupled with increases in PrEP and SSP uptake by 2027.

Results: Scenario B was more effective in reducing incidence in 2035 (9.1 new infections per 100,000) than Scenario C (12.1) compared with baseline Scenario A (13.3) (Table). The combined Scenario D resulted in only slight improvements (8.4 new infections per 100,000) compared to Scenario B. All scenarios reduced IRRs, but only Scenario E eliminated incidence disparities by 2035, with respective IRRs of 0.9 and 1.1 among the H/L and Black populations.

Conclusion: With no changes, disparities in IRR will persist through 2035. Eliminating r/e disparities in the continuum-of-care by 2027 can reduce, but not eliminate, incidence disparities by 2035. Prevention-based interventions are less effective than continuum-based interventions in reducing both overall...
incidence and r/e incidence disparities; and provide only small added benefit when supplementing continuum-of-care intervention parity. Elimination of r/e incidence disparities by 2035 is only possible if Black and H/L populations reach highest possible care and prevention levels by 2027.

### Table: 2035 incidence per 100,000 persons and 2035 incidence rate ratios

<table>
<thead>
<tr>
<th>Race/ethnic group</th>
<th>A: Baseline</th>
<th>B: CAB only</th>
<th>C: Prevent</th>
<th>D: CAB + Prevent</th>
<th>E: Max reach</th>
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<tr>
<td>Overall</td>
<td>10.3</td>
<td>9.1</td>
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<td>8.4</td>
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<td>Black</td>
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<td>Hispanic/Latino</td>
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</tr>
</tbody>
</table>

**2035 incidence rate ratio**

- Baseline to Black: 6.5
- Baseline to Hispanic/Latino: 4.1
- Baseline to Other: 4.1

### 1229 Cost-Effectiveness of Differentiated HIV Treatment Delivery in Sub-Saharan Africa: A Modeling Study

**Methods:** We adapted two pre-validated mathematical models (EMOD-HIV and Synthesis) to project health outcomes (disability-adjusted life years, DALYs) and costs (2021 USD) of DSD relative to SoC from 2022 to 2062, with a 3% discount rate. We covered four settings: South Africa/EMOD, Malawi/EMOD, Zambia/EMOD, and SSA low- and middle-income countries (LMIC)/Synthesis; and three DSD modalities for people with HIV aged 15+: community adherence groups (CAG), urban adherence groups (UAG), and home ART delivery (HomeART). Retention was defined as consistent engagement in care with no loss to follow-up (i.e., missing for > 28 days since last scheduled visit) or death. Model inputs were sourced from published literature. The effectiveness of DSD was modeled as percentage increases in annual retention rates, with values set at 25% for CAG, 38% for HomeART, and 50% for UAG for all settings. Country-specific costs included SoC and DSD visits, medications, and lab tests. We calculated incremental cost-effectiveness ratios (ICERs) of DSD versus SoC from the healthcare perspective, as compared to CE thresholds ($590-$3525/DALY for EMOD-HIV and Synthesis; and $6,135 and $21,782 at 15% usage in 10 years for EMOD-HIV and Synthesis, respectively). Three independent age- and risk-stratified HIV transmission models (EMOD-HIV, Synthesis, and Thembisa) were calibrated to epidemiological data for South Africa: Expanding PrEP usage to 5, 10, or 15% of the sexually active population via leveraging CAB-LA to supplement and increasingly replace TDF-FTC within 5 or 10 years and sustaining initiations until 2042 was simulated and compared to status quo PrEP use. Use by risk exposure varied by model. Effects were assessed in disability-adjusted life years (DALYs) over 50 years. Costs incorporated PrEP provision, outpatient antiretroviral therapy provision, and HIV-related inpatient costs by CD4 count. The potential annual total delivery cost of CAB-LA was varied from 1 to 50 times that of TDF-FTC ($US124). Costs and DALYs were discounted at 3%/year. An empirically estimated CE threshold of US$3,767/DALY was utilized.

### 1230 Model Comparison of the Cost-Effectiveness of Long-Acting Cabotegravir for HIV PrEP in South Africa

**Methods:** Three independent age- and risk-stratified HIV transmission models (EMOD-HIV, Synthesis, and Thembisa) were calibrated to epidemiological data for South Africa. Expanding PrEP usage to 5, 10, or 15% of the sexually active population via leveraging CAB-LA to supplement and increasingly replace TDF-FTC within 5 or 10 years and sustaining initiations until 2042 was simulated and compared to status quo PrEP use. Use by risk exposure varied by model. Effects were assessed in disability-adjusted life years (DALYs) over 50 years. Costs incorporated PrEP provision, outpatient antiretroviral therapy provision, and HIV-related inpatient costs by CD4 count. The potential annual total delivery cost of CAB-LA was varied from 1 to 50 times that of TDF-FTC ($US124). Costs and DALYs were discounted at 3%/year. An empirically estimated CE threshold of US$3,767/DALY was utilized.

### Results:** All models showed diminishing CE with higher PrEP use, at least partly because higher usage reaches more people with lower risk. Expansions in 5 vs 10 years typically had similar CE, with Thembisa showing the largest differences in favor of 5 years. On average, 5% usage in 5 years had the best CE and 15% in 10 the worst. With Thembisa, the median CE for 5% in 5 years was $605/DALY if CAB-LA is delivered at cost parity with TDF-FTC, and $49,617/DALY at 50-fold costs, compared to $1,076 and $72,147, respectively, if 15% PrEP usage is reached within 10 years. Compared to Thembisa, the EMOD-HIV and Synthesis models assumed more risk-informed PrEP delivery, modeled lower HIV incidence declines without CAB-LA, and had higher continuing TDF-FTC provision. Both projected expanding PrEP use with CAB-LA to be cost-saving if delivered at cost parity with TDF-FTC, and $49,617/DALY at 50-fold costs, compared to $1,076 and $72,147, respectively, if 15% PrEP usage is reached within 10 years. Compared to Thembisa, the EMOD-HIV and Synthesis models assumed more risk-informed PrEP delivery, modeled lower HIV incidence declines without CAB-LA, and had higher continuing TDF-FTC provision. Both projected expanding PrEP use with CAB-LA to be cost-saving if delivered at cost parity with TDF-FTC under all scenarios. At 50-fold costs, median estimates were $3,206 and $6,845/DALY at 5% PrEP usage in 5 years and $6,135 and $21,782 at 15% usage in 10 years for EMOD-HIV and Synthesis, respectively.

### Conclusion:** Three independent models predict expanding PrEP coverage with CAB-LA would be cost-effective if delivered at a cost near that of TDF-FTC, and highlight the importance of reaching people with high risk for CE at higher prices.
1231 Cost-Effectiveness of a Urine Tenofovir Point-of-Care Assay for ART: Adherence Feedback in Namibia

Ntombizodwa M. Nyoni,1 Sigal Maya,1 Jacques Kamangui2, Daniella Mouton2, Assegid Mengistu1, Matthew A. Spinelli,1 Pearl Kaimugogo,1 Gram Mundtani,2 Steven Y. Hong,1 Jim Kain1, Peter Minchella,1 Monica Gandhi1, Leonard T. Bikinesi4

1University of California San Francisco, San Francisco, CA, USA; 2Ministry of Health and Social Services, Windhoek, Namibia; 3Namibia Institute of Pathology, Windhoek, Namibia, 4Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Virolologic failure (VF) in patients on TLD often results from non-adherence given dolutegravir’s high resistance barrier. Real-time adherence measurement could improve counseling, help patients achieve virological suppression (VS), reduce viral load (VL) tests, and promote cost-effective (CE) adherence interventions. In Namibia, we found that a new point-of-care (POC) urine-based tenofovir (TFV) assay-based intervention increased VS to 90% among those with VF on TLD after 3 months of WHO-recommended enhanced adherence counseling (EAC). These results contrast with an overall 7% VS rate worldwide for those on ART as estimated by the UNAIDS July 2023 report.

Methods: We modeled the health and cost impacts of two possible implementation scenarios with the urine assay over a year, comparing them to the standard of care (SoC) involving monthly EAC alone for VF, with VL tests every 3 months. Scenario 1 involved monthly TFV POC testing and EAC with a VL test after 3 consecutive positive POC tests; scenario 2 further restricted the use of VL testing to patients with no record of VS throughout the intervention period and an assumption that those who achieved VS remained adherent for a year. In 2020, the cost of VL and TFV POC testing was $63 and $51.33, respectively, for both SoC and the two scenarios. The cost of EAC ranged from $1.17 to $1.92 based on duration of session. Baseline ART adherence and VS data were obtained from the clinical study and UNAIDS. We assumed no patients would drop out of care.

Results: When following SoC guidelines, in a hypothetical cohort of 100 patients with VF on TLD, EAC alone increases VS to 71% at a total cost of $22,348. Scenario 1, in which POC tests were used to determine eligibility for VL tests, saved a total of 46 VL tests with a cost-savings of $2000, and resulted in 97% adherence and 90% VS. Scenario 2, where VL testing was not performed after VS was achieved, averted another 242 VL tests and an additional $15,190 (Table). It was assumed this large reduction in VL testing would not impact overall VS rates.

Conclusion: The study evaluates the cost and cost-effectiveness of incorporating a POC urine TFV test into counseling for ongoing viroemia on TLD; the intervention was less expensive and improved health compared to SoC. An upcoming clinical trial will compare SoC to EAC to urine assay counseling in a large sample with further CE analysis with hopes of eventual wide implementation of the urine assay worldwide to reduce VF, viral resistance, and transmission.

Table: Comparison between 100 patients with virolologic failure on TLD receiving standard-of-care adherence counseling versus adherence counseling based on a point-of-care urine tenofovir assay

<table>
<thead>
<tr>
<th></th>
<th>Cost$</th>
<th>Cost-savings</th>
<th>Patients with viral suppression (%)</th>
<th>Difference in patients with VS</th>
<th>Cost-effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard care</td>
<td>$27,348</td>
<td>$1,174</td>
<td>71</td>
<td>18</td>
<td>8.5%</td>
</tr>
<tr>
<td>Intervention with VL re-testing after suppression</td>
<td>$26,211</td>
<td>$2,027</td>
<td>90</td>
<td>18</td>
<td>8.5%</td>
</tr>
<tr>
<td>Intervention with no additional VL testing after suppression</td>
<td>$10,001</td>
<td>$11,990</td>
<td>90</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Assumed counseling cost $5.77 hour and those who achieve VS would remain adherent to ART.

1All costs in 2020 USD. 2Less costly and improves health outcomes.

1232 Increased Medicare Spending Among Beneficiaries With HIV in the 12 Months Prior to Death

Jose F. Figueroa,1 Giara E. Duggan1, Jessica Phelan2, Florence Eben1, Parastara Kasai2, Luke Angel1, Ken K. Althoff3, E.J. Orav1, Emily P. Hyle1

1Harvard TH Chan School of Public Health, Boston, MA, USA; 2Massachusetts General Hospital, Boston, MA, USA; 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 4Brigham and Women's Hospital, Boston, MA, USA

Background: Medicare spending tends to be disproportionately higher among beneficiaries in their last year of life. We currently lack empirical data on how spending differs among Medicare beneficiaries with HIV in the last 12 months of life compared with Medicare beneficiaries with HIV who survive 12 months.

Methods: Using a 20% yearly sample of Medicare fee-for-service claims data (2016-2018), we identified beneficiaries with HIV aged 65 and older with Part D (prescription) coverage. We examined mean annual Medicare spending among beneficiaries with HIV who survive the calendar year compared with mean spending in the 12 months prior to death among beneficiaries with HIV who died, stratifying results by age group. After 1% winsorization, we calculated mean total spending, as well as mean spending across seven subcategories: direct medical treatment of HIV (excluding drugs); HIV-associated conditions and infections; mental health disorders; other medical spending; total drug spending; spending on antiretroviral therapy (ART); and spending on other drugs.

Results: The study sample included 5,601 beneficiaries with HIV who survived the study period, and 706 beneficiaries who died. Mean annual Medicare spending was substantially higher among decedents in the 12 months prior to death compared to people who survived, with the largest difference observed among beneficiaries aged 65-69 ($126,969 vs. $55,662). Mean annual spending was higher among decedents with HIV (compared with survivors) across most subcategories, except total drug spending. Beneficiaries of all age groups who died had lower spending on ART ($23,256-$1,532 vs. $30,650-$24,159) with the greatest decrease among older beneficiaries.

Conclusion: In a national study of older Medicare beneficiaries with HIV and Part D, beneficiaries in their last year of life incurred substantially higher spending compared to survivors, particularly among younger beneficiaries. In contrast, ART spending declined markedly in the last year of life, especially among older beneficiaries, which suggests ART cessation at the end of life.

These findings have important implications as the HIV population grows older, and a greater number of people with HIV receive healthcare coverage through the Medicare program at the end of life.

Mean Medicare spending over 12 months among beneficiaries with HIV and Part D who died vs. Beneficiaries with HIV who survived, 2016-2018

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Died vs. Survivors</th>
<th>Total Spending</th>
<th>$25,197 (N=706)</th>
<th>$15,190 (N=1000)</th>
<th>$10,000 (N=1500)</th>
<th>$5,000 (N=2500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 65-69</td>
<td>Died</td>
<td>Direct Medical Treatment of HIV (excluding drugs)</td>
<td>$13,143</td>
<td>$6,184</td>
<td>$6,957</td>
<td>$7,820</td>
</tr>
<tr>
<td></td>
<td>Survivors</td>
<td>HIV-Associated Conditions and Infections</td>
<td>$8,087</td>
<td>$4,040</td>
<td>$4,857</td>
<td>$5,720</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mental Health Disorders</td>
<td>$2,016</td>
<td>$1,016</td>
<td>$1,016</td>
<td>$1,016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other Medical Spending</td>
<td>$7,020</td>
<td>$3,500</td>
<td>$3,996</td>
<td>$3,996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Drug Spending</td>
<td>$25,256</td>
<td>$15,200</td>
<td>$16,000</td>
<td>$18,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spending on ART</td>
<td>$10,977</td>
<td>$5,950</td>
<td>$6,479</td>
<td>$7,065</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spending on Other Drugs</td>
<td>$10,977</td>
<td>$5,950</td>
<td>$6,479</td>
<td>$7,065</td>
</tr>
</tbody>
</table>

1TFV-associated conditions and infections as defined in Figueroa et al. Health Affairs 2022.

1233 Estimated Costs of Eliminating Medicaid Prior Authorizations for Antiretrovirals in Washington State

Matthew Golden1, Ryan Taketomo2, Ryan Pistoresi2

1University of Washington, Seattle, WA, USA; 2Washington State Health Care Authority, Olympia, WA, USA

Background: Medicaid is the largest insurer of persons living with HIV (PLWH) in the U.S. Some Medicaid programs use prior authorizations (PAs) to limit drug expenditures. In 2023, WA State eliminated PAs for antiretrovirals (ARVs).

Methods: We modeled the impact of PA elimination on HIV treatment and pre-exposure prophylaxis (PrEP) costs, 2023-2027. All models used 2021 net drug costs reflecting costs minus rebates. PrEP models assumed a 10% annual increase in the number of PrEP users and evaluated the impact of annual declines in the relative percentage of PrEP users on TDF/FTC with compensatory increases in TAF/FTC use; some models assumed the percentage of PrEP users on cabotegravir (CAB) would increase 1% annually. We modeled 5 scenarios: 1) Base – no change in the percentages of PrEP users on TDF/FTC and TAF/FTC and no CAB; 2) Stable change – 3.5% decrease in TDF/FTC and no CAB; 3) Accelerated change – 7% decrease in TDF/FTC and no CAB; 4) Stable change + CAB – 3.5% decrease in TDF/FTC and 1% annual increase in CAB; 5) Accelerated change + CAB – 7% decrease in TDF/FTC and 1% annual increase in CAB. For HIV treatment, we assumed a 2% annual increase in the number of PLWH on ART. Our Base Model assumed no change in the percentage of PLWH on different drugs. Our No PA Model assumed: 1) 18% annual relative increase in the percentage of PLWH on bicaptegravir/TAF/FTC; 2) the percentage of people on tenofovir/FTC taking TAF/FTC (vs. TDF/FTC) increases 9%; annually; 3) CAB/riparivirine increases 1% annually; 4) no change in darunavir/cobicistat; and 5)
use of other ART drugs decline in proportion to their 2022 use. To place costs in context, we estimated the number of PLWH who might be housed using money required to meet new ART costs; this estimate reflects local 2023 costs for emergency or temporary housing with 5% annual inflation.

**Results:** The 5-year cost of PrEP in our Base Model was $28.7 million. Elimination of PAs increases that cost by $28.2 to $57.8 million. The annual cost of PrEP in 2027 was $9.5 to $20.1 million more in no PA models than the base model. Changes in HIV treatment will result in $63 million in new costs over 5 years. In our most costly scenario, elimination of PAs will cost $121 million over 5 years. This cost would pay for 5 years of housing for 817 PLWH, which exceeds the total number of unhoused PLWH in King County, WA.

**Conclusion:** Elimination of Medicaid PAs will likely result in substantial new costs. Changes in drug formulary should consider opportunity costs.

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**Figure:** Estimated cumulative and incremental cost associated with changes in PrEP (A) and HIV treatment (B) over 5 years.

<table>
<thead>
<tr>
<th>Cost (millions)</th>
<th>Incremental Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Model</td>
<td>Stable increase TAP/PTC</td>
</tr>
<tr>
<td>Base Model</td>
<td>Accelerated increase TAP/PTC</td>
</tr>
<tr>
<td>Base Model</td>
<td>Stable increase TAP/PTC + CAB</td>
</tr>
<tr>
<td>Base Model</td>
<td>Accelerated increase TAP/PTC + CAB</td>
</tr>
<tr>
<td>Base Model</td>
<td>5 year costs</td>
</tr>
<tr>
<td>Base Model</td>
<td>Incremental cost</td>
</tr>
<tr>
<td>No PA Model</td>
<td>Stable increase TAP/PTC</td>
</tr>
<tr>
<td>No PA Model</td>
<td>Accelerated increase TAP/PTC</td>
</tr>
<tr>
<td>No PA Model</td>
<td>Stable increase TAP/PTC + CAB</td>
</tr>
<tr>
<td>No PA Model</td>
<td>Accelerated increase TAP/PTC + CAB</td>
</tr>
<tr>
<td>No PA Model</td>
<td>5 year costs</td>
</tr>
<tr>
<td>No PA Model</td>
<td>Incremental cost</td>
</tr>
</tbody>
</table>

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**Implementing Long-Acting ART in a Community Health Center: Insights and Early Outcomes**

**David Fessler,** Erin Kelley, Rachel McLaughlin, Erin Loubier, Robert Bangert, Tasliym Adams, Amanda Fuchs, Lexie Alves, Jessica Estrada, Jonathon Rendina, Sarah Henri

**Whitman-Walker Health, Washington, DC, USA Presenting Author: Dr David Fessler**

**Background:** In the current era of universal antiretroviral treatment (ART), health systems have the dual challenge of a growing number of people living with HIV and on ART who are also receiving chronic, life-long treatment for non-communicable diseases. Current evidence suggests that multi-month dispensing (MMD) for HIV can maintain at least equivalent clinical outcomes to conventional care and reduce costs, but little evidence exists when integrating MMD for multiple conditions. We examined the cost-effectiveness of integrated MMD for people living with HIV and hypertension.

**Methods:** Using an age and sex-specific hybrid decision tree and Markov state-transition model, we constructed a 100,000-person simulated population cohort who may develop HIV and hypertension and initiate treatment at clinics in South Africa over a 10-year time horizon. We assessed the incremental costs and effectiveness of MMD versus conventional care from a health system perspective under different conditions of care seeking, eligibility, and uptake of MMD for clinically stable patients. Model inputs were sourced from previously published literature. MMD was defined as reducing the frequency of clinic visits by increasing the number of medications dispensed to stable patients at each visit from 3 to 6 months. For the integrated MMD, we assumed that comorbid patients receive both HIV and hypertension drugs at the same facility on the same day.

**Results:** Our study demonstrates that integrated MMD for HIV and hypertension in South Africa can avert between 0.08 and 0.11 DALYs and save between $166 and $208 health systems costs per patient per year. Across all scenarios, HIV prevalence, care seeking level, mortality rate and LTFU rate were key drivers in DALYs averted; HIV prevalence, outpatient costs per visit and ART cost were key drivers in health systems cost. Overall, greater MMD integration, higher care seeking and a greater proportion of well-controlled patients in care led to greater cost savings or better (lower) ICER values.

**Conclusion:** Integrated MMD is likely cost-saving (or highly cost-effective at ≤$30 per DALY averted) in various care-seeking scenarios and proportions of patients in care. Scale-up of MMD strategies is most effective when multiple conditions are addressed simultaneously. The benefit of integrated MMD may be greater than what we have estimated here through the potential for greater care-seeking and disease control among patients already in care with less LTFU and fewer deaths through high-quality care.

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**1235 Implementing Long-Acting ART in a Community Health Center: Insights and Early Outcomes**

**David Fessler,** Erin Kelley, Rachel McLaughlin, Erin Loubier, Robert Bangert, Tasliym Adams, Amanda Fuchs, Lexie Alves, Jessica Estrada, Jonathon Rendina, Sarah Henri

**Whitman-Walker Health, Washington, DC, USA Presenting Author: Dr David Fessler**

**Background:** Long-acting injectable Cabotegravir-Rilpivirine (CAB-RPV LAI) offers people living with HIV (PLWH) a safe and effective alternative to daily oral ART. In 2021, Whitman-Walker Health, a Federal Qualified Health Center (FQHC), began scaling up implementation of CAB-RPV LAI in appropriate patients.

**Methods:** A multidisciplinary working group developed a protocol for providers and a workflow to streamline processes involving insurance authorizations, coding/billing, and patient and medication tracking. Most patients were consistently virologically suppressed at baseline, consistent with FDA guidance and trial protocols. However, with early promising data in treating non-suppressed PLWH (Gandhi et al., CROI 2023), carefully selected viremic patients were also offered treatment, using a monthly dosing schedule. These were generally PLWH who were struggling with adherence but who maintained consistent care engagement, and who were verified to have no relevant resistance mutations. We conducted a cross-sectional analysis comparing virologic suppression in patients on CAB-RPV LAI with patients prescribed oral ART, recognizing the presence of selection bias. The two groups were similar across age, sex, gender identity, insurance status, and baseline CD4 count.

**Results:** To date, we have 133 PLWH receiving CAB-RPV LAI, who have received a total of 715 doses of which 96% have been given within the appropriate treatment window. Navigators track to ensure injection visits are scheduled at the correct time intervals and work with medical providers to reschedule quickly for missed appointments. Most CAB-RPV LAI patients (86%, 114/133) had a VL <50 copies/mL on most recent assay, compared to 74% (2367/3176) of PLWH receiving oral HIV medication, X2 (1, N=3309) = 8.7, p<.01. Twenty-one patients were started on CAB-RPV LAI with a VL ≥ 50 copies/mL who had a VL drawn at least one month after their initial dose. This group’s median baseline VL was 100 copies/mL, and 76% (16/21) have subsequently achieved VL <50 copies/mL. Of the five patients who remained non-suppressed, median VL has dropped from 140 copies/mL to 60 copies/mL.

**Conclusion:** We successfully developed a robust cohort of PLWH on CAB-RPV LAI in an FQHC setting. These patients have maintained viral suppression at a high rate. We also provide further evidence suggesting efficacy of CAB-RPV LAI in non-suppressed PLWH who struggle with adherence to oral ART. Research focused specifically on this at-risk population is urgently needed.
1237 Associations Between Interest in Oral vs Long-Acting Injectable ART Among US Women With HIV

Morgan M. Philbin, Tara McCormick, Lauren F. Collins, Margaret Pereyra, Carbin Platamone, Anandi N. Sethi, Jarred C. Cohens, Tracey Wilson, Catalina Ramirez, David B. Hanna, Stephen J. Gange, Asli Ramas, Bani Tamraz, Lakshmi Goparaju and Maria L. Alcайд

1University of California San Francisco, San Francisco, CA, USA; 2Columbia University, New York, NY, USA; 3Emory University, Atlanta, GA, USA; 4Johns Hopkins Hospital and Hospital of Cook County, Chicago, IL, USA; 5State University of New York Downstate Medical Center Downstate Medical Center, Brooklyn, NY, USA; 6University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 7Albert Einstein College of Medicine, Bronx, NY, USA; 8The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 9University of Alabama at Birmingham, Birmingham, AL, USA; 10Georgetown University, Washington, DC, USA; 11University of Miami, Miami, FL, USA

Background: Long-acting injectable (LAI) ART has the potential to improve suboptimal medication adherence and health outcomes for people with HIV, yet studies of patient interest and acceptance are mixed and may differ by sex. We surveyed U.S. women to examine factors associated with preferences for LAI vs oral ART.

Methods: From Sept. 2020- Nov. 2021, we administered a cross-sectional survey to 1,078 women with HIV across MACS/WIHS Combined Cohort Study sites in Atlanta, GA; Birmingham, AL; Jackson, MI; Bronx, NY; Brooklyn, NY; Chapel Hill, NC; Chicago, IL; Miami, FL; San Francisco, CA; and Washington, DC. The survey assessed demographic characteristics and ART modality preferences. Multinomial logistic regression assessed factors associated with preference for LAI vs oral ART vs undecided, controlling for age, education, race/ethnicity, income, adherence and site.

Results: Median age was 54, the majority of women were Black (72%) and Hispanic (13%), and most (58%) had an annual income of ≥$52K. Over one-third (37%) of women finished more than high school while 88% reported ≥95% oral ART adherence. In the sample, 43% preferred LAI ART, 36% oral ART, and 21% were undecided. In adjusted models, women who reported ≥95% oral ART adherence (relative risk (RR): 0.39; CI: 0.24-0.63) and older age (RR: 0.98 per year; CI: 0.97-1.00) were less likely to prefer LAI ART vs oral ART. Compared with women in Miami, those in other sites (RR: 2.12-3.05) preferred LAI to oral ART. Comparing women who preferred LAI ART vs being undecided, women with ≥95% oral ART adherence were less likely to prefer LAI ART (RR: 0.58; CI: 0.33-0.99) whereas Hispanic women were more likely (RR: 2.30; CI:1.01-4.87). When comparing oral ART vs undecided, women whose racial category was 'other' were more likely to prefer oral ART (RR: 3.32; CI:2.17-5.46) whereas women whose income was ≥$52K were less likely to prefer oral ART (RR: 0.58, CI: 0.39-0.86). Women in sites other than Miami (RR: 0.26-0.32) were less likely to prefer oral ART vs undecided.

Conclusions: In a geographically diverse cohort of US women, we found a preference for LAI ART vs oral ART, while about 20% remained undecided. Findings suggest potential differences in ART preference by adherence, age, site, income and race/ethnicity. To maximize the benefits of LAI ART and ensure equitable access to treatment for all women across the US and globally, it is critical to tailor education and support around LAI ART that is specific to each patient’s unique needs and experiences.
ART and characterized their demographics, clinical characteristics, and HIV care outcomes.

**Methods:** LAI ART recipients were identified by screening both prescribing and dispensing records using a combination of medication name, RxNorm, and National Drug Code.

**Results:** A total of 234 LAI ART recipients were identified: 56.8% female, mean age 44.9, 51.3% non-Hispanic Black, 20.9% non-Hispanic White, 19.7% Hispanic, 78.2% on Medicaid and 4.7% on private insurance. About 20% of LAI ART users did not have two or more visits at least 90 days apart in the year before LAI ART initiation (i.e., were not engaged in care). Lifetime substance use disorder diagnoses included 18.8% with cannabis use disorder, 13.7% with cocaine use disorder, and 8.5% with opioid use disorder. After the initiation of LAI ART, 53.8% exclusively used LAI ART, 15% concurrently used other oral ART, and 31.2% potentially switched back to oral ART (i.e., receiving oral ART after last record of LAI ART). Most people (68.4%) had 2+ records of LAI ART injections, among them the average time gap between injections is 50.6 days (SD 45.2) and more than half had at least one time gap greater than 65 days. Of the 54 people with HIV viral load test results after the initiation of LAI ART, only 1 record of virologic failure (viral load >200 copies/ml) was observed.

**Conclusion:** Our study demonstrates that people with suboptimal care engagement and substance use disorder were not excluded from the treatment with LAI ART. While LAI ART is considered a complete regimen for HIV, a small proportion of individuals concurrently received other oral ART, possibly due to personalized treatment plans or bridging treatment. Our study reveals a likely noteworthy discrepancy between the current guidelines, which suggest a two-month interval between LAI ART injections, and the observed common prolonged time gap between injections. Despite limited evidence for optimal LAI ART adherence, virologic failure remains rare among LAI ART users.

### 1239 Interest in Long-Acting Injectable PrEP Among Transgender Women in the United States


The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, Brigham and Women’s Hospital, Boston, MA, USA, Duke University, Durham, NC, USA, ‘Callen-Lorde Community Health Center, New York, NY, USA, University of Miami Miller School of Medicine, Miami, FL, USA, Whitman-Walker Health, Washington, DC, USA, VHW Healthcare, London, United Kingdom, Harvard Medical School, Boston, MA, USA, ‘Duke Global Health Institute, Durham, NC, USA, VHW Healthcare, Durham, NC, USA, VHW Healthcare, London, UK

**Background:** Long-acting injectable (LAI) PrEP is a highly efficacious HIV prevention tool. Among communities with high HIV burden, such as transgender women (TW) in the United States (US), uptake among those at risk of acquiring HIV could potentially reduce new infections and contribute to ending the HIV Epidemic efforts. However, a lack of data on LAI PrEP interest among TW in the US has limited scientific understanding of the potential impact of LAI PrEP scale-up on new infections within TW communities. Thus, our objective was to identify correlates of interest in LAI PrEP among a large sample of TW.

**Methods:** Data were drawn from the LITE cohort, which followed TW with risk factors for HIV acquisition in the eastern and southern US. Participants who completed 12-month surveys between March 2019 and September 2021 (corresponding to the period between establishing LAI PrEP efficacy and prior to FDA approval) were asked about their interest in using LAI PrEP. We estimated crude and adjusted prevalence ratios for LAI PrEP interest with sociodemographic characteristics, healthcare access indicators, prior PrEP experience, and PrEP indication correlates using Poisson regression with robust variance.

**Results:** Among 867 TW, 23% reported they were very interested in LAI PrEP, 22% somewhat interested, 19% neutral, 7% somewhat uninterested, 12% very uninterested, and 16% unsure. In the adjusted model, interest in LAI PrEP (somewhat or very interested) was more common among TW who identified as Black, college-educated TW, and TW indicated for PrEP based on CDC guidelines (Figure). LAI PrEP interest was also more common among adherent users of oral PrEP and those who had discontinued oral PrEP, compared to PrEP-naïve participants. There were no statistically significant differences in LAI PrEP interest for young adults ages 18-24 years compared to those >25 years, those who identified as Latina/x, and those who were publicly insured or uninsured compared to those privately insured.

**Conclusion:** Interest in LAI PrEP among TW in the eastern and southern US varied by several demographic and clinical characteristics. Increased interest in LAI PrEP among Black TW, those who were PrEP indicated, and those who had previously discontinued oral PrEP underscores the need to increase LAI PrEP access for TW who express interest. Efforts to increase awareness of and access to LAI PrEP among young TW and Latina/x TW are needed to ensure equitable scale-up among TW most impacted by HIV.

### 1240 Healthcare Staff Acceptability and Feasibility of Telehealth Delivery of Cabotegravir for PrEP

**Albert Liu** 1, Alvin Kingcade 2, Toyin Nwafor 3, Bo Li 1, Neelima Jain 4, Stephen Maher 5, Ray Hsieh 1, Alison Gaudioni 1, Riya Moodley 1, Deanna Merrill 1, Lisa Petty 1, Piotr Budnik 2, Jean Van Wyk 1, Maggie Czmokowski 1, Naniesta Pirgim 1

1 San Francisco Department of Public Health, San Francisco, CA, USA, 2Bebas - Transtion to Hope, Philadelphia, PA, USA, 3VHW Healthcare, Durham, NC, USA, 4GSR, Collegeville, PA, USA, 5Aveda, Bethesda, MD, USA, 6VHW Healthcare, Brentford, United Kingdom

**Background:** Telehealth can be leveraged to support PrEP delivery; however, limited evidence exists on how it is being used with injectable PrEP. We report interim findings from the PILLAR study, a Phase IV implementation science study, on healthcare staff acceptability and feasibility of long acting cabotegravir (CAB LA) for PrEP use of telehealth, and utility of implementation supports for CAB LA delivery in the U.S.

**Methods:** 17 sites were randomized 2:1 to Dynamic (DI) or Routine (RI) implementation. DI received standard toolkits and DI received standard and enhanced toolkits and supports. Staff study participants (SSPs) completed the acceptability of intervention (AIM) and feasibility of intervention measures (FIM) for CAB LA delivery and for implementation support at Month 1 (M1; n=86) and Month 4 (M4; n=80). Change in mean FIM and AIM scores (scores averaged over four items measured as 1=completely disagree to 5=completely agree) was assessed. SSPs completed questions on the utility of implementation supports and telehealth delivery of CAB LA at M4.

**Results:** Of 86 SSPs at M1, 50% were cisgender female, 55% White, 15% Black, and 24% Hispanic, with mean age 41 (range: 23-72). SSPs reported high levels of acceptability and feasibility of CAB LA and implementation support at M1 (mean scale scores ≥4.0) and M4 (mean scale scores ≥3.9). Except for FIM for CAB LA in RI, mean scores decreased slightly over time for both arms (≤0.4). At M4, toolkits with the highest use (>40% SSPs) were found useful/very useful by over 50% of SSPs (Table). DI only toolkits and support with the highest use (>33% SSPs) were found useful by over 47% of SSPs (Table). 69% of DI SSPs found one-on-one and group facilitation support useful/very useful. Of 51 DI arm SSPs at M4, 57% reported using telehealth for CAB LA delivery. Of those using telehealth, the systems used included virtual visits (83%), online appointment scheduling (52%)/reminders (76%), and at home testing (38%)/injections (24%). DI SSPs found the systems very/somewhat easy to use (range: 71%-86%), very helpful/helpful (range: 71%-93%) and were very/somewhat satisfied (range: 80%-94.7%). DI SSPs reported high levels of acceptability and feasibility of telehealth delivery of CAB LA (mean scale scores ≥4.1).

**Conclusion:** Findings suggest that CAB LA telehealth delivery is acceptable and feasible. Scaling telehealth and implementation resources may support efficient integration of CAB LA into care and user adherence and retention on CAB LA. The figure, table, or graphic for this abstract has been removed.
1241 Insurance Type Drives Cabotegravir Delays: Real-World Long-Acting PrEP Outcomes in the Midwest US

Aniruddha Hazra1, John Schneider1, Megan Murray2, Jean Williams1, Drew Halbur2, Catherine Creticos3

1University of Chicago, Chicago, IL, USA, 2Howard Brown Health Center, Chicago, IL, USA

Background: Long-acting cabotegravir (CAB-LA) is the first FDA-approved injectable agent for HIV PrEP. However, limited data exist on its use in a real-world setting. We describe clinical characteristics and outcomes of CAB-LA for PrEP at a federally qualified health center and the largest PrEP clinic in the Midwest US.

Methods: We conducted a single-center retrospective cohort study of all patients without HIV who received at least one dose of CAB-LA at Howard Brown Health Center between July 1, 2022 and December 31, 2023. Demographics and clinical characteristics were collected; associations between delays in initiation as well as therapy interruption and discontinuation were assessed by logistic regression.

Results: A total of 270 patients met the inclusion criteria with median age 33, 80.4% cisgender men, 54.1% White, 23.7% Hispanic, 60.4% with private insurance, and 29.6% residing in a high HIV vulnerability community area. The majority (90.4%) had been on oral PrEP in the past, 72.2% transitioned directly from oral PrEP, 2.2% had a CAB oral lead-in, and 31.1% had a BMI >30kg/m². Patients experienced a median delay of 24 days (IQR 11-51) between a provider initiating a CAB-LA prescription and their first injection, 41.9% of patients experienced delays >30 days. The median number of CAB-LA doses received was 4 (range, 1-12); 8.1% experienced at least one delayed or missed injection and 10% of patients discontinued CAB-LA of which 25.9% were placed on an oral PrEP regimen after discontinuation. While on CAB-LA, 67% had HIV screening by viral load assay at every injection visit; 49.6% had STI screening at the recommended 4-6 month intervals with 26.3% testing positive for an STI during the study period. Insurance status was the only variable associated with delays in initiation. Compared to patients with private insurance, those without insurance were significantly more likely to experience delays while those with public insurance were significantly less likely to experience delays >30 days in initiating CAB-LA (OR 1.97 vs 0.60, p=0.03).

Conclusion: CAB-LA for PrEP was successfully implemented at a large urban community health center. Most patients experienced delays in initiation which were associated with insurance type; interruptions and discontinuation of injections were less common without any significant associations. More work is needed to support patients and health systems with challenges specific to long-acting agents, particularly regarding insurers and payors.

1242 Rapid Long-Acting Injectable PrEP Implementation in a Vulnerable Urban Safety Net Clinic Population

Ezra Bisom-Rapp, Christina Camp, Jon Oskarsson, Mary Shieks, Matthew A. Spinelli, Francis Mayorga-Munoz, Anthonia Chimeme, Monica Gandhi, Sarah Puryear

University of California San Francisco, San Francisco, CA, USA

Background: Despite effective oral PrEP barriers to uptake and adherence persist, including stigma, substance use (SU), housing/food insecurity, and mental health issues. Long-acting cabotegravir (CAB) PrEP is highly efficacious and may address barriers for patients with adherence challenges, but uptake has been low and real-world data is lacking.

Methods: We describe a CAB demonstration project at Ward 86, an urban safety-net clinic in San Francisco serving vulnerable patients with high rates of homelessness, mental illness, and SU living with or at risk for HIV. Patients were initiated on CAB via a structured process of provider referral and multidisciplinary review and monitored for on-time injections. Patients were offered low-barrier, multidisciplinary care with rapid PrEP initiation. We report CAB PrEP program evaluation data from chart extraction and PrEP tracking logs.

Results: We initiated 30 participants on CAB PrEP: 70% cisgender men, 13% cisgender women, 7% transgender women, 10% nonbinary people. Median age was 39 years (range 21-63); 10% of participants were Black, 30% Hispanic, 7% Asian/Pacific Islander, 3% multi-racial. Of the 30, 20% were homeless; 23% were unsheltered housed. All participants had public insurance (93%) or were uninsured (7%). Active SU disorder was documented in 67%; 53% had at least 1 serious mental illness. We administered 184 injections during the study period, with a median of 6 injections/person (range 1-10). Median time from CAB referral to first injection was 14 days (range 0-111). The median duration of follow-up was 271 days (IQR 87-469). All patients had direct-to- inject CAB; 50% were not on oral PrEP at CAB initiation. Of 157 scheduled follow-up injections, 86% were on time, 4% early, 8% late, and 2% missed/not done. Of late or missed/not done injections, 47% were ≥1 month late, of which 43% were covered by oral PrEP bridging. CAB was discontinued by 3 participants for risk change (1), loss-to-follow-up (1), and transfer of care (1). All remain HIV-negative.

Conclusion: This demonstration project shows that a rapid start CAB PrEP program serving patients with numerous psychosocial stressors is feasible and has high retention. Delivery of CAB via a low-barrier, comprehensive care model may expand uptake to high-risk, adherence-challenged populations.

1243 High Acceptability of Long-Acting Injectable PrEP and ART Among MSM and PWID in India

Allison M. McFall1, Talia A. Leeb1, Jiban B. Baishya2, Ashwini Kedar2, Archit Sinha3, Aylur K Srikrishnan4, Sunil Sahas Solomoni2, Gregory M. Lucas1, Shruti H. Mehta1

1The Johns Hopkins University, Baltimore, MD, USA, 2The Johns Hopkins University, New Delhi, India, 3YR Gattonede Center for AIDS Research and Education, New Delhi, India, 4YR Gattonede Center for AIDS Research and Education, Chennai, India

Background: Long-acting injectable PrEP (LA-PrEP) and ART (LA-ART) hold significant potential to overcome challenges of daily adherence to oral modalities. Their population-level impact requires acceptability and uptake by populations with the most need, such as people who inject drugs (PWID) and men who have sex with men (MSM) in low/middle income settings.

Methods: We used respondent-driven sampling to accrue samples of PWID and MSM in India Nov 2022-Aug 2023 (3 PWID sites; 2 MSM sites; ~750/site).

Participants were ≥18 years old and reported injection drug use in the prior 2 years (PWID) or sex with a man in the prior year (MSM). Participants completed a survey and blood draw. Those reporting a prior HIV diagnosis were asked questions about LA-ART and those without a prior diagnosis were asked about LA-PrEP. We present acceptability and correlates of LA-PrEP and LA-ART using multilevel logistic regression models by modality and population separately.

Results: 2250 PWID and 1502 MSM were enrolled. PWID HIV prevalence was 41%, of whom 45% were on ART and 34% suppressed (<150 copies/mL). MSM HIV prevalence was 21%, of whom 65% were on ART and 75% suppressed. Of those not reporting a prior HIV diagnosis, 10/14% (PWID/MSM, respectively) reported no/very little chance they would be willing to get injections to prevent HIV and 65/78% a very good chance. Main reasons for LA-PrEP unwillingness among PWID were inconvenience of injections and low risk perception; among MSM, reasons were injection pain and cost. Among PWID, women and those homeless were less willing to take LA-PrEP and those with more sexual partners were more willing; injection behaviors were not associated (Table). Among MSM, those with more partners were less willing to take LA-PrEP; unprotected sex was not associated. Of those with a prior HIV diagnosis, 3/2% (PWID/MSM, respectively) reported no/very little chance they would willing to take LA-ART and 83/89% reported a very good chance. Among PWID, those injecting daily were more willing to take LA-ART but those viremic were less likely. Among MSM, those with more partners and viremic were more willing. ART use was not associated with LA-ART willingness for either population.

Conclusion: There was high interest in using LA-PrEP and LA-ART among populations that experience a disproportionate HIV burden and barriers to HIV treatment engagement. However, there remain vulnerable subgroups for which these may not be appropriate and other prevention and treatment approaches will be required.
1244 Gender Affirmation and Incentives for Long-Acting PrEP: Stated Preferences of Transgender Adults
Marta Wilson-Barthes1, Arjie Restar2, Emerson Ducis3, Don Operario1, Timothy Souza3, Omar Galarraga1
1 Brown University, Providence, RI, USA, 2 University of Washington, Seattle, WA, USA, 3 Emory University, Atlanta, GA, USA
Background: Transgender and nonbinary (trans) people face a disproportionately high HIV risk, yet adherence to pre-exposure prophylaxis (PrEP) remains low. Conventional approaches to PrEP programming have not sufficiently engaged trans populations. Gender affirmation and conditional economic incentives could help improve the uptake of PrEP, but user-centered approaches are needed to inform the optimal design of trans-specific PrEP programming.
Methods: We conducted a discrete choice experiment among 385 trans adults in Seattle/King County, Washington State to inform the optimal design of a conditional economic incentive (CEI) program that would provide free long-acting injectable PrEP (LA-PrEP) and gender-affirming care. We used a best-elicitation method where respondents were first asked to select their best option from three hypothetical choice profiles (Program A, Program B, or No Program), and then to select their second-best option from the remaining two profiles. We used a rank-ordered mixed logit model for main results, and estimated respondents’ marginal willingness-to-accept each program attribute.
Results: We find the optimal program design would: (1) deliver incentives in cash, (2) confirm LA-PrEP adherence via blood testing, (3) provide counseling in-person, and (4) provide co-prescriptions for injectable gender-affirming hormones. From a maximum yearly incentive amount of $1,200, respondents were willing to forgo up to $795 to receive incentives in cash (instead of voucher) and up to $567 to receive injectable rather than oral hormones. As shown in the figure, the probability of choosing a hypothetical program over no program waned as adults aged (>40 years) and as annual income increased (>57,000/year).
Conclusion: Conditional economic incentives may be effective for improving LA-PrEP adherence among trans adults who are younger and have fewer financial resources. A randomized trial is needed to confirm the validity of the DCE for predicting actual program uptake.

1245 Access to Long-Acting Injectable ART Through State AIDS Drug Assistance Programs
Lauren C. Zalla1, Tim Horn2, Catherine Lesko1
1 The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2 National Association of State and Territorial AIDS Directors (NASTAD) in Washington, DC, USA
Background: Long-acting injectable (LAI) antiretroviral therapy (ART) may address barriers to treatment adherence among people with HIV. Due to cost and operational issues, low-income individuals, including clients of the federal Ryan White HIV/AIDS Program (RWHP), may face greater challenges in accessing LAI ART. Of 1.09 million people with diagnosed HIV in the US, 301K (28%) access treatment through state AIDS Drug Assistance Programs (ADAPs) funded by the RWHP. State ADAPs are required to cover at least one drug from each therapeutic class of HIV antiretrovirals (ARVs). They are not required to cover long-acting formulations of drugs from existing therapeutic classes, such long-acting cabotegravir/raltegravir (Cabenuva). We sought to determine the extent of access to Cabenuva through state ADAPs, and to describe the population of ADAP clients without access to Cabenuva.
Methods: Data on state ADAP medication formularies were collected by the National Association of State and Territorial AIDS Directors (NASTAD) in January 2023. Data on the characteristics of ADAP clients were obtained from the 2020 RWHP ADAP Annual Client-Level Report. Data on the characteristics of all people with diagnosed HIV were obtained from the US Center for Disease Control and Prevention’s AtlasPlus Tool. We compared the characteristics of ADAP clients in states with and without ADAP coverage of Cabenuva, and of ADAP clients vs. non-clients in states without ADAP coverage of Cabenuva.
Results: In contrast to the 2 oral ARVs most recently approved by the US Food and Drug Administration, which were listed on 92–98% of state ADAP medication formularies in January 2023, Cabenuva (approved in January 2021) was covered by 78% of state ADAPs. Nearly two thirds (64%) of the 56,020 ADAP clients in states without ADAP coverage of Cabenuva were living at or below the federal poverty level, compared to 43% of the 221,539 ADAP clients in states that did cover Cabenuva. In states that did not cover Cabenuva, ADAP clients were more likely than non-clients to be Black (36% vs. 34%) or Hispanic (30% vs. 26%).
Conclusion: Gaps in coverage of Cabenuva affect large numbers of people who access ART through state ADAPs, and disproportionately affect low-income and racially minoritized people with HIV. States should consider expanding access to Cabenuva, including working to reduce supply and payment chain barriers, to promote equitable access to LAI ART. Future work should examine access to Cabenuva using patient-level data.

1246 Effect of Changes in Script Renewal Period on HIV Viral Non-Suppression Among Insured South Africans
Gabriela E. Patten1, Mary-Anne Davies2, Gary Maartens2, Naomi Falb2, Andreas Haar1
1 University of Cape Town, Cape Town, South Africa, 2 University of Bern, Bern, Switzerland
Background: Evidence is needed to inform antiretroviral therapy (ART) delivery models which allow people with HIV to attend health facilities less frequently. While COVID-19 lockdown measures were in place, South Africa temporarily changed the validity of “repeat” prescriptions for ART from 6 to 12 months. We evaluated the effect of these changes on HIV viral non-suppression in the private health sector in South Africa.
Methods: We analysed routine laboratory and pharmacy claim data between November 1, 2019, and November 30, 2022 from Aid for AIDS (AfA), a large South African medical insurance scheme. Adults living with HIV over the age of 15 years who had received ART for ≥3 months were included. We conducted an interrupted time-series analysis comparing rates of viral non-suppression (viral load [VL] ≥50 copies/mL) during the following periods: November 1, 2019 to April 23, 2020, November 1, 2019 to September 25, 2021 when the 12-monthly script renewal period was re-instated. Monthly suppression rates were modelled using binomial generalized linear regression models. We measured the slope change in suppression rates between adjacent time periods by including an interaction term between time and a binary indicator for the 12-monthly script renewal period in the model. Inverse probability weighting was used to account for changes in viral load testing during the period.
Results: The study population included 73884 in January 2019, increasing to 76321 in May 2020 and 72026 in November 2022. The proportion with unknown VL increased during the study. (Figure A) Compared to the period prior to lockdown, the odds of viral non-suppression was lower among patients when 12-monthly script renewal was in place (adjusted odds ratio [aOR] 0.99, 95% confidence interval [CI] 0.99-0.99, p<0.001). When 6-monthly script renewal was re-introduced the odds of viral non-suppression increased (aOR 1.02 [95% CI 1.02-1.02], p<0.001). (Figure B)
Conclusion: Our results show longer script renewal period was associated with slight improvements in viral suppression. Measures implemented during COVID-19 to ensure continued access to chronic medication in South Africa provided a unique opportunity for evidence to guide efficient ART delivery models involving less frequent visits to healthcare providers without negatively affecting treatment outcomes.
1247 High Viral Load Suppression Rates Among People Living With HIV Receiving Multi-Month Dispensing

Andrew Mugisa1, Christopher Bwanika2, Josephine Nakakande3, Jane Nakaweesi4, Peter Amutungire1
1Makerere University, Kampala, Uganda; 2Makerere University College of Health Sciences, Kampala, Uganda

Background: The World Health Organization (WHO) recommended differentiated service delivery (DSD) with multi-month dispensing (MMD) as a way of improving care for people living with HIV (PLHIV). Uganda adopted MMD in the 2020 consolidated guidelines for the prevention and treatment of HIV and AIDS in Uganda. We sought to determine the likelihood of viral load (VL) non-suppression among PLHIV on MMD in Mubende region, central Uganda.

Methods: A cross-sectional review of Uganda Electronic Medical Records (Uganda EMR) System program data was conducted at 10 purposively selected high volume facilities in eight districts of central Uganda. We included PLHIV who had one recent documented VL result from January 2022 to February 2023 at last visit. Data on history of advanced HIV disease status and frequency ofVL monitoring were retrieved from information over the 13 months of patient care. Logistic regression models were used to assess the effect of MMD on VL suppression among PLHIV. Analysis included PLHIV switched to MMD after being determined stable i.e., on ART for more than 6 months, with a suppressed VL, absence of Advanced HIV Disease (AHD) and not pregnant or not lactating for less than 6 months. PLHIV who didn’t fall in these criteria were considered as non-MMD.

Results: We reviewed records for 19,455 PLHIV for whom 67% were women. Median age of the participants was 37 years (Interquartile range (IQR) 29-47) with 10% of the participants being <20 years. 97% (18,960/19,455) of the participants had a suppressed VL of less than 1,000 copies/mL. Median duration on ART for clients initially not on MMD but switched to MMD was 74 months (IQR: 45-105) and 52 months (IQR: 21.5-89.5) for non MMD. Participants on MMD had significantly lower odds of VL non-suppression compared to those on non-MMD (Adjusted Odds Ratio (aOR) of 0.09 (95% CI: 0.07-0.12). However, participants on MMD but with a history of AHD and those on MMD on 2nd line ARV regimen had increased odds of attaining VL non-suppression, aOR of 4.71 (95% CI: 2.82-7.86) and 2.31 (95% CI: 1.69-3.16) respectively compared to those with no history of AHD and those on 1st line ARV regimen.

Conclusion: PLHIV on MMD with history of AHD and PLHIV on MMD on second line ARVs have increased likelihood of VL non-suppression and therefore need to be monitored closely monitored. An in-depth qualitative study may be helpful to understand other factors that contribute to increased odds of suppression among clients on MMD.

1248 HIV Care Retention in 3 Multi-Month ART Dispensing: A Retrospective Cohort Study in Mozambique

Anna Saura-Lázaro1, Orvalho Augusto2, Sheila Fernández-Luis3, Elisa López-Varela4, Laura Fuente-Soro5, Dulce C. Bilia6, Milagre Tovela7, Nello Macacaun8, Paula Vaz9, Aleny Couto10, Carmen Bruno11, Denise Nanciche12
1Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain; 2Centro de Investigación en Saúde de Manhiça (CISM), Maputo, Mozambique; 3Fundaçao Arviel Glazier Contra o SIDA Pediatras, Maputo, Mozambique; 4Programa Nacional de Controle de ITS, HIV/SIDA, Maputo, Mozambique; 5Dirección Provincial de Salud, Maputo, Mozambique

Background: In Mozambique, enrolment of people living with HIV (PLHIV) into three multi-month dispensing (3MMD) of antiretroviral therapy (ART) has been on continuous clinical stability and ≥6 months on ART. As a COVID-19 control measure, the required time on ART was shortened to ≥3 months. We assessed the effect of 3MMD on the retention in care of PLHIV considering their time on ART prior to enrolment.

Methods: A retrospective cohort study of routine patient data was conducted including PLHIV ≥10 years who started ART between January 2018 and March 2021 in Manhiça District. PLHIV were followed until December 2021. Attrition included lost to follow-up, death and transfer out. Kaplan-Meier estimates were used to calculate cumulative retention in care probabilities after ART initiation. Cox proportional-hazards models, with inverse probability weights of ART initiation enrolment, were used to compare attrition between 3MMD and monthly ART dispensing, stratifying by “established enrollers” (<6 months on ART) and “early enrollers” (<6 months on ART). Analyses were stratified by adolescents and youth (AYLHIV) (10-24 years) and adults (≥25 years).

Results: A total of 7,378 PLHIV were included, 25% AYHIV (86% female and median age of 21) and 75% adults (57% female and median age of 35), of whom 59% and 62% were enrolled in 3MMD. Over 90% of early enrolments occurred after COVID-19 measures. Median follow-up time was 11.3 (IQR: 5.7-21.6) and 10.2 (IQR: 4.8-20.9) months in ART and adults, respectively. Both early-established and early 3MMD enrollers showed higher retention rates compared to individuals on monthly dispensing (p-value <0.001, Figure). Likewise, the attrition risk was lower for both established (aHR AYLHIV=0.65; 95%CI: 0.54-0.78 and aHR adults=0.50; 95%CI: 0.44-0.56) and early enrollers (aHR AYLHIV=0.70; 95%CI: 0.58-0.85 and aHR adults=0.63; 95%CI: 0.57-0.70). Lastly, among individuals in 3MMD, male gender (aHR=1.30; 95%CI: 1.18-1.44) and receiving care in a medium/low-volume healthcare facility (aHR=1.18; 95%CI: 1.03-1.34) increased attrition risk. Conversely, longer ART time before 3MMD enrolment (aHR AYLHIV=0.93; 95% CI: 0.92-0.94 per one-month increase) and age ≥45 years (aHR=0.77, 95% CI: 0.67-0.89) reduced risk.

Conclusion: 3MMD improves retention in care compared to monthly dispensing among established and early enrollers, although to a lesser extent among the latter. To reap maximum benefits, shortening the required time on ART prior to 3MMD enrolment should be accompanied by additional support services. The figure, table, or graphic for this abstract has been removed.

1249 Using Best-Worst Scaling Experiments to Identify Profiles of Client & Provider Preferences in Zambia

Njeawka Mukamba1, Musunge Mutabule2, Markman Koloka2, Noelle Le Toumeau2, Kombatende Sikombe1, Sandra Simbeza1, Anjali Sharma1, Laura K. Beres2, Jake M. Pry1, Carolyn Bolton1, Elvin H. Geng2, Isukanjji Sikazwe3, Aikoje Mody2
1Centre for Infectious Disease Research in Zambia, Lusaka, Zambia; 2Washington University in St Louis, St Louis, MO, USA; 3The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Background: People living with HIV (PLWH) who reengage after falling out of care remain high risk for repeat disengagement, but few tailored reengagement strategies exist to support sustained engagement in this diverse. We used best-worst scaling (BWS) experiments to understand client and health care worker (HCW) preferences for features of reengagement strategies.

Methods: We conducted BWS surveys among clients returning to care after being >30 days late to an appointment without ART as well as HCWs at 4 public HIV clinics in Lusaka, Zambia. Participants identified the statements about varied reengagement care features and services that they most and least preferred across multiple choice sets. For both groups, we used multinomial-
logit models to quantify relative preference scores, scaled from 0–100, and latent class analysis to identify unique preference profiles for strategies.

**Results:** We administered BWS surveys among 144 PLWH returning to care (55% female, median age 38 [IQR 19–81]) and 171 HCWs (8% clinical officers, 16% nurses, 46% lay HCWs, 30% other). Overall, clients preferred rapid ART reinitiation (normalized score 14.9), longer ART refills (12.0), kind reception at return (11.1), empathy for care challenges (10.9), timely viral load (VL) monitoring (10.2) and coordinating drug pick-ups at other clinics when travelling (9.8). HCWs prioritized timely VL monitoring (11.2), longer drug refills (8.8), flexible ART access after unexpected life events (ULES) (8.8), kind receptions (8.6), and rapid ART restart (8.3). We identified 3 unique preference profiles for each group: 46% of clients preferred improved reengagement experiences (e.g., rapid ART restart, kind receptions), 27% desired easier ART access for travel/ULES (e.g., longer refills, travel drug pick-ups, flexible ART access), and 27% sought more convenient clinical experiences (e.g., longer refills, rapid ART restart, flexible ART access). Amongst HCWs, 41% prioritized improving client experience (e.g., flexible appointments, kind receptions), 30% focused on ART access and client outreach (e.g., longer refills, community ART delivery), and 27% prioritized clinical needs (e.g., rapid ART restart, VL monitoring) (Figure).

**Conclusion:** Although clients and HCWs had similar overall preferences, there are unique preference profiles that differentially prioritize improvements to client experience, access to medications, or addressing clinical needs. Multipronged, tailored reengagement strategies are needed to address the needs for all returning PLWH.

**Appendix 1:**

![Figure 1: Preferences for Services to Improve Care after Reengagement by Client (Closed)](image)

**1250 Automated Condom Distribution for HIV Prevention: Mwanza, Tanzania, 2021-2023**

Aafke Kineono, Julie Franks, Emmanuel Mihayo, Macdonald Mahiti, Joyce Thomas, Oresto Munishi, Omari Musimi, John Khamene, Koku Kuhubwa Kauzura, Nyangode Nyangode, Mbaraka Amuri, Haruka Maruyama

1ICAP at Columbia University, Dar es Salaam, United Republic of Tanzania; 2ICAP at Columbia University, New York, NY, USA; 3Ministry of Health, Mbabane, Eswatini; 4Centers for Disease Control and Prevention, Atlanta, GA, USA; 5Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania

**Background:** In Tanzania, factors such as fear of stigma associated with getting condoms, concerns about cost, stock outs, and limited outlets beyond the health facility make it hard for people to access condoms. Barriers to access limit condom use among key and vulnerable populations (KVPs) highly impacted by HIV, including adolescent girls and young women (AGYW), female sex workers (FSW), men who have sex with men (MSM), and people who inject drugs (PWID). Automated dispensing technology is a standalone unit consisting of simple electro-mechanical systems to automate the entire distribution process. The unit’s interactive digital display, aesthetically pleasing and ergonomically designed, improves the user experience, and increases access to condoms. We describe the use of automated digital dispensing machines in community settings to increase access to condoms.

**Methods:** We installed six machines at hotspots in Mwanza City frequented by KVPs. Peer outreach workers from the KVP communities were trained on use of the machines and sensitized peers in surrounding communities. Machines dispensed free condoms to people with access codes. Peers distributed unique personal identification numbers to KVP for up to 15 free condoms per day. Non-KVP clients received single-use access for up to three condoms per day. Machines captured user demographics and number of condoms dispensed through a self-guided questionnaire using the machine’s touch screen. All data was stored in a secured server to ensure information security and confidentiality.

**Results:** Between October 2021 and June 2023, a total of 545,581 condoms were distributed to 6,572 KVP; users had a mean age of 29 (range: 25–29) years. Among users, 68% were female, and 77% (n=418,512) of condoms were collected by females. In addition, 57% of condoms were retrieved by FSWs; 15% by AGYW; 14% by MSM; and 14% by PWID. A mean of 93 (range: 62–210) condoms were accessed per female user compared to 61 condoms (range: 4–112) per male user. Machines were accessible 24 hours, 7 days a week. Machine peak access days were over weekends with peak access times between 4 p.m. to 6 p.m.

**Conclusion:** The proportion of female clients that accessed condoms is generally high. Machines installed in key locations and incorporated with community-based, peer-led approaches can expand condom access beyond health facilities and to times when most health facilities were closed. Technology-based condom distribution solutions are crucial in addressing barriers to condom access in KVPs.

**1251 Cost-Effectiveness of an Agricultural Intervention Among Adults With HIV and Their Children in Kenya**

Assurah W. Elly, Eliy Weke, Pauline Wekesa, Rachel Burger, Lila Sheira, Mocello Adrienne, Edward Frongillo, Lisa Butler, Sheri Weiser, Craig Cohen, Jim Kahn, Elizabeth Bukusi, Stanley Shade

1Kenya Medical Research Institute-UCSF Infectious Disease Research Training Program, Kisumu, Kenya; 2University of California San Francisco, San Francisco, CA, USA

**Background:** Agricultural interventions to address food insecurity have been shown to improve HIV health outcomes through nutritional, mental health, and health behavior pathways, but little is known about their costs and cost-effectiveness

**Methods:** We estimated costs and incremental cost-effectiveness of implementing a multisectoral agricultural intervention (Shamba Maisha) compared to control within a cluster-randomized trial in 8 matched health facilities during 2016–2019. Shamba Maisha included loans to purchase a human-powered irrigation pump, fertilizer, seeds and pesticides, and provision of training in sustainable agriculture and financial literacy. Participants were adults in HIV care at participating clinics who were followed for 24 months. We estimated cost per person using site visits, conversations with study coordinators, microcosting techniques, a time-and-motion personnel study, and administrative record review. Costs were categorized into capital goods, personnel costs, and recurrent goods and services. We observed effects of the intervention on food insecurity, depression, and social support among participants and length/height for age among their young children (aged 6-23.9 months). Results from the study were translated into disability-adjusted life years (DALYs) averted. We estimated the incremental cost per DALY averted among participants and their young children in the intervention compared to those in the control arm.

**Results:** Participants included 720 adults with HIV (366 intervention, 354 control). Results were also assessed among 207 young children (95 intervention, 112 control). Overall, the incremental cost of implementation of Shamba Maisha compared to control was $642 per person ($841 intervention, $199 control). This included an added cost per person of $529 for capital goods, $387 for personnel salaries and benefits, $22 for recurring goods, and $24 for recurring services. The intervention averted an estimated 0.158 DALYs per person, including 0.027 DALYs for decreased food insecurity, 0.053 for decreased depression, 0.044 for increased social support, and 0.014 for greater length/height for age among young children. The overall incremental cost-effectiveness ratio was $5407 per DALY averted.

**Conclusion:** The Shamba Maisha multisectoral agricultural intervention was cost-effective based on the WHO threshold of three times the annual GDP per
1252 Integrated HIV+ NCD Care in Community Microfinance venues: Harembarre Cluster Randomized Trial Results

Becky Genberg,1 Jon Steinigrmson2, Juddy Wachira1, Catherine Kafu3, Marta Wilson-Barthez4, Sonak Pastakia4, Dan N. Tran5, Jamil A. Said6, Rajesh Vedanthan7, Suzanne Goodrich8, Paula Braithwaite9, Youjin Lee1, Joseph Hogan1, Omar Galarraga3
1The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2Brown University, Providence, RI, USA, 3Marist University, Edison, NY, USA, 4Academic Model Providing Access to Healthcare, Eldoret, Kenya, 5Purdue University, West Lafayette, IN, USA, 6Temple University, Philadelphia, PA, USA, 7New York University Langone Medical Center, New York, NY, USA, 8Indiana University, Indianapolis, IN, USA, 9University of Toronto, Toronto, Canada

Background: In Sub-Saharan Africa, distance to health facilities, vertical care delivery, increased burden of non-communicable diseases (NCDs), and limited socioeconomic resources are barriers to patients maintaining HIV viral suppression. The Harembarre cluster randomized trial tested a differentiated care model delivering HIV+NCD care within microfinance (MF) groups of people living with HIV in western Kenya.

Methods: Fifty-seven MF groups (n=855 participants) were randomized in a 1:1 ratio to receive integrated community-based (ICB) care or standard facility-based care (SOC). The ICB intervention included: (1) clinical care visits during MF group meetings inclusive of clinical consultations, NCD management, distribution of antiretroviral therapy (ART) and NCD medications; (2) support for ART adherence; and (3) facility referrals as needed. Primary outcome was viral suppression (<400 HIV-RNA/mL before January 1, 2023; <200 HIV-RNA/mL on or after January 1, 2023) at 18 months. Effectiveness was estimated as the difference in viral suppression at 18-months comparing the two trial arms using a doubly robust generalized estimating equation accounting for dropout and adjusting for baseline viral load. The primary outcome from both trial arms were also compared to an additional n=300 propensity-matched controls receiving usual care (UC) alone.

Results: The sample was 52 years of age, 75% female and viral suppression at baseline was 94%. There was no intervention effect on viral suppression at 18-months when comparing MF+ICB to MF+SOC (odds ratio, OR: 1.26, 95% Confidence Interval CI: [0.90, 1.60], p-value =0.23). There was an increase in viral suppression in the MF+ICB (OR: 2.16, 95% CI [1.48, 3.19], p-value <0.001) and MF+SOC (OR: 1.42, 95% CI [1.05, 1.92], p-value = 0.023), compared to the matched UC group.

Conclusion: Among MF groups, those who received integrated HIV+NCD care did not have statistically higher viral suppression compared to SOC. This may be due to high viral suppression at baseline in both arms. However, improvements in viral suppression among MF group participants compared to matched usual care users suggests that microfinance improves HIV treatment outcomes among patients in rural Kenya, yet additional research is necessary to understand the mechanisms for how MF improves viral suppression. Differentiated care models for addressing multilevel barriers to the maintenance of HIV viral suppression may be more effective if socioeconomic barriers are mitigated.

1253 Persistence on Contraception and PrEP in Hair Salons in South Africa

Ingrid V. Bassett1, Joyce Yan2, Sabina Govere3, Shabile Shezi4, Lungle M. Ngcobo2, Shroiti Sagar5, Jana Jarolimova1, Dani Zionts1, Christina Psaros6, Nduvoido Dube7, Robert A. Parker1
1Massachusetts General Hospital, Boston, MA, USA, 2AIDS Healthcare Foundation, Durban, South Africa

Background: Young women have high HIV incidence and risk for unintended births in sub-Saharan Africa. Women congregate regularly in hair salons; these may be useful community settings for providing HIV prevention and family planning. Our objective was to assess PrEP and contraceptive persistence following dispensing in hair salons in South Africa.

Methods: We conducted a pilot randomized trial to evaluate uptake and persistence of a nurse-supported intervention offering PrEP (TDF-FTC) and contraception (oral/injectable) in 5 salons in urban KwaZulu-Natal. Women could start PrEP and/or contraception at the initial visit or opt in at a later visit. We defined persistence as one additional visit within 6 months with continued treatment (PrEP, contraception, or both). We assessed the association of PrEP persistence among intervention participants using contingency tables. Factors assessed included age, self-perceived risk of HIV, partner ≥5y older, primary sex partner having other partners, intimate partner violence, curable STI at enrollment, and persistence on contraception.

Results: Among 125 participants in the intervention salons, median age was 26y (IQR 22.29-29. 93 (75%) reported visiting the salon at least every 2 months; 34 (27%) were taking hormonal contraception at enrollment. 25 (28%) described themselves as having moderate or greater chance of getting HIV in the next year and 35 (32%) think their primary sex partner has other partners. 46 (37%) initiated PrEP during the study; among the 40 returning for at least 6 months of follow-up, 17 (43%) persisted. 39 (21%) opted for oral contraception and 77 (62%) for injectable contraception; among the 94 with at least 6 months follow-up, 66 (70%) persisted on contraception. Persistence on salons-based PrEP was associated with age ≥25y (RR: 3.45 [95% CI: 1.16, 24.3]) and intimate partner violence (2.57 [1.24,4.91]). PrEP persistence was also related to no/low perceived risk of HIV and contraceptive persistence (RR undefined).

Conclusion: Young women in South Africa found receipt of HIV prevention services and family planning in a hair salon acceptable over time, with persistence for contraception (70%) greater than for PrEP (43%). Factors related to PrEP persistence include age ≥ 25y, intimate partner violence, a low or no perceived risk of HIV, and persistence on contraceptives. Hair salons are a novel venue for delivering long-term sexual reproductive health services, however, a menu of PrEP delivery methods may be required to support persistence.

Table 1. Association of Potential Predictors with PrEP Persistence

<table>
<thead>
<tr>
<th>Level</th>
<th>Persistencea</th>
<th>Persistenceb</th>
<th>Relative Risk (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td>25 or older</td>
<td>25 or under</td>
<td>18% (15-21)</td>
<td>23% (24-43)</td>
</tr>
<tr>
<td>Self-perceived risk of HIV</td>
<td>None or low</td>
<td>None or low</td>
<td>15% (12-18)</td>
<td>17% (19-34)</td>
</tr>
<tr>
<td>Partner ≥5y older</td>
<td>No</td>
<td>Yes</td>
<td>40% (32-50)</td>
<td>60% (45-71)</td>
</tr>
<tr>
<td>PrEP use</td>
<td>No</td>
<td>Yes</td>
<td>54% (45-62)</td>
<td>64% (53-73)</td>
</tr>
<tr>
<td>Intimate partner violence</td>
<td>No</td>
<td>Yes</td>
<td>86% (78-92)</td>
<td>No</td>
</tr>
<tr>
<td>Curb HIV in next 2 years</td>
<td>No</td>
<td>Yes</td>
<td>39% (30-48)</td>
<td>75% (67-82)</td>
</tr>
<tr>
<td>Presence on contraceptives</td>
<td>No</td>
<td>Yes</td>
<td>87% (74-92)</td>
<td>No</td>
</tr>
</tbody>
</table>

* Entries are % (Number persisting/ N in category)
** Fisher’s Exact test

1254 Persistence in Care After PrEP Initiation Through a Community-Based Mobile Clinic

Susanne Doblecki-Lewis1, Ariana L. Johnson2, Katherine King4, Katherine Kloes3, Gillianne Narcisse4, Mario Stevenson1
1University of Miami - University of Miami (Miami, FL, USA) Preventing Author; Dr. Susanne Doblecki-Lewis University of Miami- University of Miami (Miami, FL, USA)

Background: PrEP can reduce HIV infections substantially when implemented effectively. Miami, the area of the United States (US) with the highest rate of new HIV infections, has significant structural, social, and logistic barriers to PrEP care. Alternative care models, such as mobile clinics, can increase access to PrEP. There are no available data on persistence in PrEP care through a community-based mobile clinic.

Methods: Clients sought PrEP services through one of 5 mobile sites or at the fixed site from August 2018 - March 2023 excluding March-September 2020 due to the COVID-19 pandemic. 24-week persistence was defined as at least 1 follow-up appointment within 24 weeks of initiation, and 48-week persistence as having at least 1 additional follow-up appointment between 24 and 48 weeks. Cox proportional hazards models were used to estimate adjusted Hazard Ratio (aHR) of risk factors for discontinuation of care by 48 weeks by gender, race, ethnicity, insurance status, and visit site.

Results: 919 clients initiated PrEP before March 2022. Clients were primarily self-reported male (86.8%), white (69.7%), Hispanic (74.6%), insured (50.6%), and initiated services at the mobile clinic (52.2%). Overall persistence on PrEP was 56.7% at 24 weeks and 41.5% at 48 weeks. Individuals who were uninsured, identified as male, and initiated services in the mobile clinic were more likely to continue PrEP (HR:1.20, p=0.01; HR:2.02, p<0.01; HR:1.68, p<0.01, respectively). Overall persistence in care (including visits for other sexual health services) was 76.2% to 24 weeks and 55.7% at 48 weeks. Individuals who identified as male, and those who initiated services at the mobile clinic had increased continuation (HR:1.51, p=0.02; HR:2.21, p<0.01, respectively).

Conclusion: Persistence in PrEP and sexual health care is improved for those initiating services in a community-based mobile clinic compared with a fixed clinic with otherwise identical services, staff, and barrier- lowering strategies.
In our analysis, uninsured clients had improved persistence on PrEP compared with those who were insured, suggesting that our no-cost service model with aggressive navigation to available assistance programs can successfully overcome barriers due to insurance coverage. Race and ethnicity were not associated with persistence in our analysis. Persistence among women initiating PrEP in both the mobile and fixed clinics was decreased. Future research to assess the potential role of mobile clinics in PrEP delivery are warranted.

**1255 Availability of Onsite Substance Use Disorder Services in HIV Facilities by Urbanicity in the US**

Kashif Iqbal, Preetam A. Cholli, Yunfeng Tie, Stacy Crim, Jesse G. O’Shea, John Weiser, Sharoda Dasgupta
Centers for Disease Control and Prevention, Atlanta, GA, USA

**Background:** HIV outbreaks related to injection drug use continue to be reported in the U.S., including in rural communities, where accessing HIV care services and substance use disorder (SUD) services may be challenging. We describe availability of onsite SUD services at HIV care facilities attended by a representative sample of people with HIV (PWH) in the U.S. and the percentage of persons receiving these services by urbanicity.

**Methods:** We analyzed 2021 survey data from 514 HIV care facilities representing all facilities providing care to a national probability sample of U.S. adults with HIV. Facility data on availability of SUD services, including SUD treatment (SUDT), medication-assisted treatment (MAT), and syringe services (SS), were collected. Data were linked to Medical Monitoring Project (MMP) participant data from 2019. Weighted percentages of characteristics at the facility and patient-level were reported by urbanicity, as defined by the Rural-Urban Continuum Code codes. Urbanicity was categorized as follows: counties in metropolitan areas of ≥1,000,000 people (large metro); counties in metropolitan areas of 250,000–1,000,000 people (medium metro); and counties in metropolitan areas of <250,000 people (small metro/nonmetro).

**Results:** Of facilities attended by MMP participants, 72% were in large metro counties, 15% were in medium metro counties, and 13% were in small metro/nonmetro counties. The percentage of PWH receiving HIV care was highest in large metro counties (73%), followed by medium (18%) and small/nonmetro counties (9%). The availability of onsite SUD services at HIV facilities varied by urbanicity (large metro, medium metro, and small/nonmetro), with SUDT at 31%, 31%, and 21%; MAT at 29%, 30%, and 20%; and SS at 9%, 3%, and 8% of facilities, respectively. (Figure 1A). Corresponding percentages of persons attending facilities with onsite SUD services varied by urbanicity (large metro, medium metro, and small/nonmetro), with SUDT at 35%, 42%, 23%; MAT at 36%, 22%, and 17%; and SS at 7%, 5%, and 7%, respectively (Figure 1B).

**Conclusion:** Onsite SUD service availability is limited at HIV care facilities and may vary by urbanicity, with potentially lower access in less urban counties. Ensuring access to SUD services could help limit risk of HIV outbreaks related to injection drug use, a national priority for achieving goals for the Ending the HIV Epidemic initiative.
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